ISSN 0077-4995

NATIONAL INSTITUTE OF GENETICS

JAPAN

ANNUAL REPORT

No. 38 1987

Published by
THE NATIONAL INSTITUTE OF GENETICS
Misima, Sizuoka-ken, Japan
1988

Annual Report of the National Institute of Genetics

No. 38, 1987

Published by
The National Institute of Genetics, Japan
1988

CONTENTS

II. Microbial Genetics

III. Immunogenetics

GENERAL STATEMENT

This report presents an outline of research activities carried out in our institute during the year 1987. The total expenditure (not including that for facilities) for 1987 FY (April 1987–March 1988) amounted to 1 billion 70 million yen, a half of which was for personnel expenses. In addition, grants-in-aid amounting to 190 million yen were rendered to selected staff members by the Ministry of Education, Science and Culture (Mombusho). Changes in personnel allotments included new positions for three members approved from April: two research staff members (*joshu*), one for the DNA Research Center and the other for the Genetic Stock Research Center, and an administrative official for the Department of Administration. Thus the total number of positions available for regular staff is 96, of which 56 are for research staff. Concerning facilities, a new building for the DNA Research Center was completed at the end of January, and two greenhouses for rice and mulberries were also completed at the end of March.

The variety and breadth of research conducted in this institute is reflected in the honors bestowed on its members, the degree of international participation in joint research and conferences and the contributions to the scientific community and the community at large made in the last year.

I am happy to note that three of our colleagues were honored in the past year. Prof. Motoo Kimura, Laboratory of Population Genetics, was awarded two prizes: the Asahi Prize for 1986 by the Asahi Shimbun Press, and the John J. Carty Award for the Advancement of Science by the National Academy of Sciences, U.S.A., for his outstanding contributions to the theoretical aspects of population and evolutionary genetics, most notably for his neutral theory of molecular evolution now widely accepted. In addition, because of his distinguished services to friendship between Japan and France through biology, he was decorated with the Chevalier de l'Ordre National du Mérite by the French Government. He was elected as an honorary member of the Genetics Society of the United Kindgom. Prof. Tomoko Ohta of the same laboratory was awarded the Weldon Memorial Prize for 1986 by Oxford University. It is given every three years to the person who has in recent years published the most noteworthy contributions to Biometric Science. Prof. Kimura also received the same

prize in 1965. Dr. Takashi Gojobori of the Laboratory of Evolutionary Genetics won an Encouragement Award from the Genetics Society of Japan for his "Studies on Molecular Evolution by Comparing the Nucleotide Sequences of Genes."

International personnel exchanges were very active in the past year. Members of our staff went abroad on 27 occasions for the purpose of presenting research results at various scientific meetings, exchanging information, and carrying out collaborative studies or field investigations; three members staying longer than 3 months. In addition, 31 scientists visited our institute from abroad, with whom information and views on recent studies were actively exchanged. Some of them delivered stimulating lectures at the Biological Symposium. Those who stayed longer than three months and carried out collaborative studies with our staff members were: Miss Pascale Barbier, Université des Sciences et Techniques du Languedoc, Montpéllier, France; Dr. Nguen Xuan Hong, Hanoi University, Vietnam; Dr. Jozefa Styrna, Jagiellonian University, Poland; Dr. Dong Sang Suh, Genetic Engineering Center, Korea Advanced Institute of Technology, and Dr. Sam-Eun Kim, Sericultural Experiment Station, Rural Development Administration, Republic of Korea; and Dr. Ling Hua Tang, Institute of Food Crops, Jiangsu Academy of Agricultural Sciences, People's Republic of China.

The past year also saw a number of changes in the staff of the institute. Dr. E. Matsunaga was reappointed on October 1 to an additional two year term as institute director. Dr. Toru Fujishima of the Laboratory of Agricultural Genetics, who had been carrying out selection experiments on the sexual maturity of Japanese quails and genetic studies on food preference in mice, left in September for Hisamitsu Pharmaceutical Company as the director of its Kyudo Safety Research Division; Dr. Masatoshi Yamamoto of the Laboratory of Cytogenetics, who had been engaging in developmental and molecular genetic studies of *Drosophila melanogaster*, was promoted in November to Associate professor of Miyazaki Medical College. On the other hand, the following three joined us: Mr. Hidenori Hayashida (postgraduate student of Kyushu University) and Dr. Hitoshi Ueda (postdoctoral fellow, National Cancer Institute, NIH, USA) were appointed as *joshu* in the DNA Research Center in April and to the Genetic Stock Research Center in October, respectively; Dr. Asao Fujiyama (research associate of the Dept. of Biochemistry and Molecular Biology,

University of Chicago) was appointed as Associate professor of the Laboratory of Human Genetics in December. Dr. T. K. Watanabe, Associate professor, was transferred in April to the Genetic Stock Research Center from the Laboratory of Evolutionary Genetics. In addition, Dr. Takeshi Seno, Saitama Cancer Center Research Institute, will join us as a professor at the Laboratory of Mutagenesis in January 1988.

We mourn the loss of two colleagues in the past year. Dr. Taro Fujii, Associate professor of the Plant Section of the Genetic Stock Research Center, died from lung cancer on May 11 at the age of 60, and Dr. Takeo Maruyama, Professor of the Laboratory of Evolutionary Genetics and Head of the DNA Research Center, died from a heart attack on December 11 at the age of 51.

Starting his scientific career in the institute in 1950, Dr. Fujii published a number of papers, under the guidance of the late Dr. Seiji Matsumura (Head of the Laboratory of Induced Mutation), in the field of radiation genetics with plants such as wheat and *Arabidopsis*. He then extended his research to the development of test systems for environmental mutagens using soybeans and maize, and more recently to a collaborative work on nitrogen fixation in the rhizosphere of rice. After being transferred to the Genetic Stock Research Center, he also made efforts to conserve various strains of higher plants, and took administrative charge of the Experimental Farm as its head.

Dr. Maruyama joined our institute in 1966 as a staff member of the Department of Population Genetics headed by Prof. Kimura, and he engaged in the mathematical treatment of various genetic problems including the stochastic process in population genetics. He published a number of significant papers on the rules governing genetic variability in geographically structured populations, of which two (Genet. Res. 15: 221–225, 1970; Genetics 70: 639–651, 1972) have been adopted in the Benchmark Papers in Genetics (Vol. 7, edited by W. H. Li, 1977). After the reorganization of the institute in 1984 he devoted himself energetically to the establishment of the DNA Data Bank of Japan (DDBJ). Thanks to his endeavor, on-line service of the data base was made available last autumn for researchers all over the country. While confronting the challenge of how we could contribute to international cooperation in this field, his sudden and unexpected death means a serious loss to the institute.

Continued improvement in the physical plant of the institute has allowed

for the expansion of research and the development of sophisticated information services that will benefit the entire scientific community. As mentioned above, an upgraded electronic computer was set up in March and on-line service of the DNA data base was started in autumn by connecting telephone circuits. As in the previous year the following works were continued by the staff of the DNA Data Analysis Section: Construction and distribution of the DNA data base including input of data published in Japanese specialty journals, the development of analytical programs, data analysis, and the issue and distribution of the DDBJ Newsletter (No. 6).

The Genetic Stock Research Center supplies, as far as possible, upon request of competent researchers in and outside of Japan, specific strains of mouse, Drosophila, *E. coli*, *B. subtilis*, etc. which are being kept in the Center. The total number of cases supplied in 1986 amounted to 247 (28 sent abroad) and the total number of strains supplied was 4773 (490 sent abroad). In cooperation with other staff, Dr. Shin-ya Iyama continued to systematize data-base information about location, characteristics, availability of distribution, etc., of a variety of experimental organisms preserved in universities and research institutes in Japan. In the past year he compiled the "Mouse Genetic Stock List Preserved in Universities and Institutes in Japan, 1987" and issued "Rice Genetics Newsletter, No. 3". These materials were distributed to interested researchers in and outside of Japan.

In our continuing effort to serve the community of Mishima, the institute held its yearly open house for the public on April 18th. Some of the research activities of each laboratory were exhibited using panels, microscopes, microcomputers, and molecular models. Scientific films were also shown. The double cherry blossoms on the campus were at their best and some 3000 visitors in the neighborhood enjoyed them. On November 14, public lectures were given at the National Science Museum in Tokyo; the titles were "Genetic study on the subspecies differentiation of the mouse and its implication in the biomedical research" by Prof. Kazuo Moriwaki and "Characteristics of codon choice pattern found for individual organisms" by Associate prof. Toshimichi Ikemura. In spite of it being a Saturday afternoon about 140 eager people from universities and institutes listened to the lectures, which were followed by rather specialized questions and lively discussion.

As reported previously, our institute was reorganized into a national center for joint use by universities in April 1984. Other inter-university

research institutes of the same category, all belonging to Mombusho, included the Institute of Statistical Mathematics in Tokyo, the Institute for High Energy Physics in Tsukuba, the Institute of Molecular Sciences, the Institute of Basic Biology, and the Institute of Physiological Sciences in Okazaki. During the past years directors of the national inter-university research institutes have deliberated with Mombusho about the plan of creating a "Postgraduate University for Advanced Studies" (tentatively named) by taking advantage of existing resources of their respective institutes. As a result, a master plan was drawn last March, and an office was set up in April in Mombusho for preparatory operation (Head: Prof. S. Nagakura, President, Okazaki National Research Institutes). An ad hoc committee started in June (Chairman: Prof. I. Tanaka, President of the Tokyo Institute of Technology), and it is anticipated that the new university will be open in October 1988. Our institute, while accepting up to six postgraduate students in genetics course every year, will represent, together with the Institute for Basic Biology and the Institute for Physsiological Sciences, the specialty of "Life Science".

As an inter-university institute we accepted, in the last year, 39 collaborative programs, 18 workshops, 9 postgraduate students, 11 research fellows and 9 scholarships from industries, and 2 entrusted research projects from private corporations. Yet, there remain a number of problems to be solved in order to achieve the reality of reform. Among other things, construction of the following facilities is of the highest priority; an RI Center, which is indispensable for the expansion of research activities on the molecular level; lodgings for visiting researchers with welfare facilities, and a second main building to accommodate laboratories for joint use by visiting professors. We are eager to do our best to accomplish the missions of our institute. We hope to have continued encouragement and support from all persons concerned.

E. Matsunaga

STAFF

Director

MATSUNAGA, Ei, D. Med., D. Sc.

Members

1. ***Department of Molecular Genetics***

ISHIHAMA, Akira, D. Sc., Head of the Department

Laboratory of Molecular Genetics

ISHIHAMA, Akira, D. Sc., Professor
FUKUDA, Ryuji, D. Med., Associate professor
FUJITA, Nobuyuki, D. Sc.
NAGATA, Kyosuke, D. Pharm.

Laboratory of Mutagenesis

SADAIE, Yoshito, D. Sc., Associate professor
INOEU, Tadashi, D. Ag.
TEZUKA, Hideo

Laboratory of Nucleic Acid Chemistry (*Guest member*)

YOSHIKAWA, Hiroshi, D. Sc., Professor
MIZUMOTO, Kiyohisa, D. Sc., Associate professor

2. ***Department of Cell Genetics***

MORIWAKI, Kazuo, D. Sc., Head of the Department

Laboratory of Cytogenetics

MORIWAKI, Kazuo, D. Sc., Professor
IMAI, Hirotami T., D. Sc., Associate professor
SHIROISHI, Toshihiko, D. Sc.

Laboratory of Microbial Genetics

YASUDA, Seiichi, D. Sc., Associate professor
NISHIMURA, Yukinobu, D. Sc.
HARA, Hiroshi, D. Sc.

Laboratory of Cytoplasmic Genetics (*Guest member*)

SUZUKI, Hideho, D. Sc., Associate professor

YONEKAWA, Hiromichi, D. Sc., Associate professor

3. *Department of Ontogenetics*

KURODA, Yukiaki, D. Sc., Head of the Department

Laboratory of Developmental Genetics

SUGIYAMA, Tsutomu, Ph. D., Professor

NAWA, Saburo, D. Sc., Professor

FUJISAWA, Toshitaka, Ph. D., Associate professor

SHIMIZU, Hiroshi, D. Eng.

Laboratory of Phenogenetics

KURODA, Yukiaki, D. Sc., Professor

MURAKAMI, Akio, D. Ag., D. Sc., Associate professor

MINATO, Kiyoshi, M. Sc.

YAMADA, Masa-Aki, D. Ag.

Laboratory of Physiological Genetics (*Guest member*)

SHIMADA, Yutaka, D. Med., Professor

IDE, Hiroyuki, D. Sc., Associate professor

4. *Department of Population Genetics*

KIMURA, Motoo, Ph. D., D. Sc., Head of the Department

Laboratory of Population Genetics

KIMURA, Motoo, Ph. D., D. Sc., Professor

OHTA, Tomoko, Ph. D., D. Sc., Professor

TAKAHATA, Naoyuki, D. Sc., Associate professor

AOKI, Kenichi, Ph. D.

Laboratory of Evolutionary Genetics

TUTIKAWA, Kiyosi, Associate Professor

GOJOBORI, Takashi, D. Sc.

Laboratory of Theoretical Genetics (*Guest member*)

MUKAI, Terumi, Ph. D., D. Sc., Professor

HORI, Hiroshi, D. Sc., Associate professor

5. *Department of Integrated Genetics*

IMAMURA, Takashi, D. Med., Head of the Department

Laboratory of Human Genetics

IMAMURA, Takashi, D. Med., Professor

FUJIYAMA, Asao, D. Sc., Associate professor

HORAI, Satoshi, D. Med.
NAKASHIMA, Hitoshi

Laboratory of Agricultural Genetics
MORISHIMA-OKINO, Hiroko, D. Ag., Professor
ENDO, Toru, D. Ag., Associate professor
SATO-HIRAOKA, Yoichiro, D. Ag.

Laboratory of Applied Genetics (*Guest member*)
WATANABE, Takeshi, D. Med., Professor

6. *Research Facilities*

Genetic Stock Research Center
MORIWAKI, Kazuo, D. Sc., Head of the Center
MIYASHITA, Nobumoto, D. Med.
WATANABE, Takao, K., D. Sc., Associate professor
UEDA, Hitoshi, D. Ag.
SANO, Yoshio, D. Ag.
NISHIMURA, Akiko, D. Ag.
IYAMA, Shin-ya, D. Ag., Associate professor

DNA Research Center
MATSUNAGA, Ei, D. Med., D. Sc., Head of the Center
IKEMURA, Toshimichi, D. Sc., Associate professor
HIROSE, Susumu, D. Sc., Associate professor
MIYAZAWA, Sanzo, D. Sc., Associate professor
HAYASHIDA, Hidenori

Experimental Farm
IYAMA, Shin-ya, D. Ag., Head of the Farm
MIYAZAWA, Akira

7. *Technical Section*

SUGIYAMA, Tsutomu, Ph. D., Chief of the Section

8. *Department of Administration*

UEMATSU, Yoshihiro, Head of the Department
UJIIE, Jun, Chief of the General Affairs Section
TANIGUCHI, Hiroshi, Chief of the Finance Section

COUNCIL

Chairman

YAMAMURA, Yuichi; Emeritus professor, Osaka University

Vice-chairman

MOROHOSHI, Seijiro; Emeritus professor, Tokyo University of Agriculture and Technology

Members (Alphabetical order)

EGAMI, Nobuo; Director, National Institute for Environmental Studies

EGAMI, Nobuo; Director, National Institute for Environmental Studies

IINO, Tetsuo; Professor, Faculty of Science, University of Tokyo

INOUYE, Eiji; Emeritus professor, University of Tokyo

NAGAKURA, Saburo; President, Okazaki National Research Institutes

NAKAI, Junnosuke; President, Hamamatsu University School of Medicine

NATORI, Reiji; Emeritus professor, Jikei University School of Medicine

NOMURA, Tatsuji; Director, Central Institute for Experimental Animals

OKADA, Tokindo S.; Director-General, Institute for Basic Biology

ONOE, Hisao; Dean, Faculty of Economics, Osaka Industrial University

OZEKI, Haruo; Professor, Faculty of Science, Kyoto University

SAKAI, Fuminori; Director, Japan Society for the Promotion of Science

SASA, Manabu; President, Toyama Medical and Pharmaceutical University

SATO, Ryo; Emeritus professor, Osaka University

TAZIMA, Yataro; Director, Institute of Silkworm Genetics and Breeding

WATANABE, Itaru; Emeritus Professor, Keio University

YAMAGATA, Hirotada; Professor, Faculty of Agriculture, Kyoto University

ADVISORY COMMITTEE

Chairman

KIMURA, Motoo; Professor, Laboratory of Population Genetics

Vice-chairman

OHBA, Shigeru; Professor, Okayama University of Science

Members (Alphabetical order)

FUKUDA, Ichiro; Professor, Faculty of Liberal Arts and Sciences, Tokyo

Women's University
MIURA, Kin-ichiro; Professor, Faculty of Engineering, University of Tokyo
MUKAI, Terumi; Professor, Faculty of Science, Kyushu University
OKADA, Masukichi; Professor, Institute of Biological Sciences, University of Tsukuba
OSAWA, Shozo; Professor, Faculty of Science, Nagoya University
SAKAGUCHI, Bungo; Professor, Faculty of Agriculture, Kyushu University
SASAKI, Motomichi; Professor, Chromosome Research Unit, Faculty of Science, Hokkaido University
TSUNEWAKI, Koichiro; Professor, Faculty of Agriculture, Kyoto University
YAMADA, Yukio; Professor, Faculty of Agriculture, Kyoto University

---

IMAMURA, Takashi; Professor, Laboratory of Human Genetics
ISHIHAMA, Akira; Professor, Laboratory of Molecular Genetics
KURODA, Yukiaki; Professor, Laboratory of Phenogenetics
MORISHIMA-OKINO, Hiroko; Professor, Laboratory of Agricultural Genetics
MORIWAKI, Kazuo; Professor, Laboratory of Cytogenetics
SUGIYAMA, Tsutomu; Professor, Laboratory of Developmental Genetics

ASSOCIATION FOR PROPAGATION OF THE KNOWLEDGE OF GENETICS

KONDO, Norio; President, Emeritus professor of Tokyo University of Agriculture
KURODA, Yukiaki; Managing Director, Professor of National Institute of Genetics
MORIWAKI, Kazuo; Managing Director, Professor of National Institute of Genetics
TAZIMA, Yataro, Manager, Director of Institute of Silkworm Genetics and Breeding
MIURA, Kin-ichiro; Manager, Professor of University of Tokyo
YAMAGUCHI, Hikoyuki; Manager, Professor of University of Tokyo
MATSUNAGA, Ei; Manager, Director of National Institute of Genetics
ISHIHAMA, Akira; Manager, Professor of National Institute of Genetics

PROJECTS OF RESEARCH FOR 1987

1. DEPARTMENT OF MOLECULAR GENETICS

Laboratory of Molecular genetics

Studies on regulatory mechanisms of gene transcription (ISHIHAMA, FUKUDA, FUJITA and NAGATA)
Studies on molecular mechanisms of transcription and replication of animal viruses (ISHIHAMA, FUKUDA and NAGATA)

Laboratory of Mutagenesis

DNA repair and radiation sensitivity in mammals (INOUE and TEZUKA)
Biochemical factors involved in cellular repair of genetic damage and induced mutagenesis (INOUE)
Radiation genetics of *Caenorhabditis elegans* (SADAIE)
Molecular mechanisms of unicellular differentiation in *Bacillus subtilis* (SADAIE)

Laboratory of Nucleic Acid Chemisry

Studies on regulatory mechanisms of chromosome replication (YOSHIKAWA)
Molecular mechanisms of RNA 5′-cap formation (MIZUMOTO)
Studies on molecular mechanisms of transcription and replication of Sendai virus (MIZUMOTO)

2. DEPARTMENT OF CELL GENETICS

Laboratory of Cytogenetics

Studies on species differentiation of mouse from cyto- and molecular genetic view points (MORIWAKI)
Immunogenetical studies on the mouse MHC (SHIROISHI and MORIWAKI)
Theoretical bases for chromosomal evolution in mammals and ants (IMAI)
Cyto- and molecular mechanism of meiotic recombination in mice (SHIRO-

ISHI, IMAI and MORIWAKI)
Cytogenetical studies on Drosophila (YAMAMOTO)

Laboratory of Microbial Genetics

DNA replication in *E. coli* (YASUDA)
Cellular division in *E. coli* (NISHIMURA and HARA)
Penicillin-binding proteins in *E. coli* (HARA and NISHIMURA)

Laboratory of Cytoplasmic Genetics

Peptidoglycan biosynthesis in *E. coli* (SUZUKI)
Studies on cytoplasmic genes during subspecies differentiation of house mouse *Mus musculus* (YONEKAWA)

3. DEPARTMENT OF ONTOGENETICS

Laboratory of Developmental Genetics

Genetic analysis of developmental mechanisms in hydra (SUGIYAMA, FUJISAWA and SHIMIZU)
Studies on transformation and cell differentiation in higher organisms (NAWA and YAMADA)
Mitochondrial DNA organization in male-sterile cytoplasms of rice (NAWA, SANO and FUJII)

Laboratory of Phenogenetics

Genetic studies on insect cells in tissue culture (KURODA and MINATO)
Developmental genetic studies on animal cells in tissue culture (KURODA)
Genetics of somatic mammalian cells in culture (KURODA)
Genetic studies on the reproduction in *Bombyx* (MURAKAMI)
Ecogenetic studies on the nerve system characters in *Bombyx* (MURAKAMI)
Studies on the male-specific killing activity of SRO in *Drosophila* (YAMADA)

Laboratory of Physiological Genetics

Electron microscopic studies on differentiation of animal cells (SHIMADA and KURODA)

Nerve network formation in *Hydra* (KIJIMA)
Genetic analysis of nervous system development in hydra (KIJIMA and SUGIYAMA)

4. DEPARTMENT OF POPULATION GENETICS

Laboratory of Population Genetics

Theoretical studies of population genetics (KIMURA, OHTA, TAKAHATA and AOKI)
Studies on molecular evolution from the standpoint of population genetics (KIMURA, TAKAHATA and OHTA)
Theoretical studies on the evolution of multigene family (OHTA)
Theory of gene genealogy (TAKAHATA)
Theoretical studies on the evolution of altruism (KIMURA and AOKI)
Population genetical studies on gene-culture coevolution (AOKI)

Laboratory of Evolutionary Genetics

Theory of population genetics and evolution (MARUYAMA)
Studies on molecular evolution (GOJOBORI)
Radiation genetics in mice (TUTIKAWA)

Laboratory of Theoretical Genetics

Theoretical and experimental studies of transposons in population of Drosophila (MUKAI)
Computer studies on the molecular evolution (HORI)

5. DEPARTMENT OF INTEGRATED GENETICS

Laboratory of Human Genetics

Molecular genetics of growth and differentiation of human blood forming cells (IMAMURA, FUJIYAMA and NAKASHIMA)
Molecular genetics of human metabolic disorders (IMAMURA, FUJIYAMA and NAKASHIMA)
Studies on DNA polymorphisms in human populations (HORAI and MATSU-

NAGA)
Mitochondrial DNA restriction analysis of non-human primates (HORAI, MATSUNAGA, HAYASAKA, SHOTAKE and NOZAWA)

Laboratory of Agricultural Genetics

Evolutionary studies in wild and cultivated rice species (MORISHIMA and SATO)
Genecological studies on pollination in rice plants (SATO and MORISHIMA)
Genetic studies on rice reserve proteins (ENDO)

Laboratory of Applied Genetics

Molecular genetics of human immune mechanisms (WATANABE)

6. RESEARCH FACILITIES

Genetic Stock Research Center

Studies and conservation of germplasm resources in rice and wheat species (FUJII and SANO)
Specificity of mutagen tolerance in higher plants (FUJII)
Exploitation of genetic ability of nitrogen fixation in Gramineae (FUJII, SANO and IYAMA)
Studies on genetic differentiation in rice (SANO)
Theoretical studies on breeding techniques (IYAMA)
Genetic studies of trees in natural forest (IYAMA)
Studies on gene transfer in Gramineae (SANO and FUJII)
Documentation of genetic stocks in Japan (IYAMA)
Studies on the management system of genetic stocks information (IYAMA)
Evolutionary genetics of *Drosophila* (WATANABE)
Molecular genetics of insect development (UEDA)
Coordination of DNA synthesis and cell division in *E. coli* (NISHIMURA)
Mapping of a whole set of cell division genes in *Escherichia coli* K12 (NISHIMURA)
Development of mouse embryo freezing system (MIYASHITA and MORIWAKI)
Genetic mechanisms for regulating tumor development in the laboratory and wild mice (MIYASHITA and MORIWAKI)

DNA Research Center

Regulatory mechanisms of gene transcription (ISHIHAMA)
DNA data analysis (MARUYAMA)
Studies on codon usage (IKEMURA)
Studies on RNA molecules of mouse brain (IKEMURA)
Database design for DNA base sequences (MIYAZAWA and HAYASHIDA)
Molecular genetics of insect development (UEDA and HIROSE)
Control of gene expression in eukaryotes (HIROSE and UEDA)

RESEARCH ACTIVITIES IN 1987

I. MOLECULAR GENETICS

Mapping of Functional Sites on Subunits of *Escherichia coli* RNA Polymerase

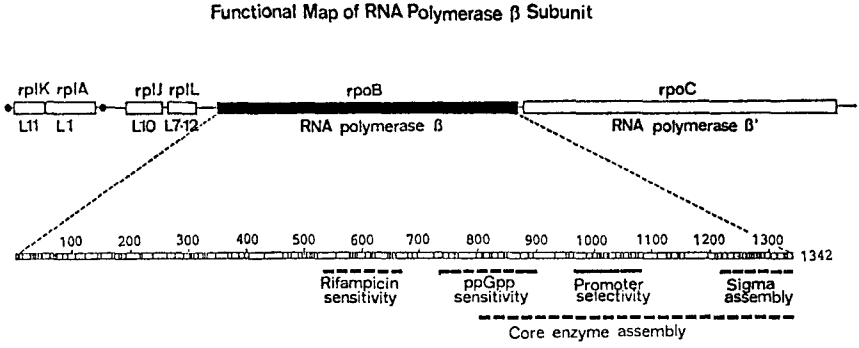
Akira ISHIHAMA, Nobuyuki FUJITA, Rei UESHIMA
and Robert E. GLASS*

A major form of RNA polymerase holoenzyme in exponentially growing cells of *Escherichia coli* is a big complex with a subunit composition of $\alpha_2\beta\beta'\sigma^{70}$. The function(s) of each subunit in transcription has been analyzed by a variefy of genetic and biochemical methods (reviewed in Ishihama, A. (1988) *Trends Genet.*, in press). In order to get a detailed understanding of the structure-function relationship, we carried out a systematic study on the mapping of functional site(s) within each subunit polypeptide.

To determine the sites on the β subunit, we used a collection of *E. coli* mutants with nonsense mutations in the *rpoB* gene encoding β subunit. By introducing various suppressor genes, variant RNA polymerases were produced, each carrying a single amino acid substitution at a defined site on β polypeptide. Results of analyses of altered functions associated with variant RNA polymerases (Glass, R. E. *et al.* (1986) *Mol. Gen. Genet.* **203**: 265–268; Glass, R. E. *et al.* (1986) *Mol. Gen. Genet.* **203**: 487–491; Glass, R. E. *et al.* (1986) *Mol. Gen. Genet.* **203**: 492–495; Ishihama, A. *et al.* (1987) *Proteins* **2**: 42–53) led us to propose a tentative functional map of the β subunit as shown in Fig. 1. To confirm this map, attempts are being made to affinity label the functional sites with substrates and ppGpp and to directly determine the binding sites for these nucleotides.

Two sigma subunits, σ^{70} and σ^{32}, carry identical sequences of 14 amino acids in length. These sequences might play an essential role in one of the functions common to the two sigma subunits. In order to identify other sigma subunits in *E. coli*, we made antibodies against a synthetic peptide

* Department of Biochemistry, Queen's Medical Centre, Nottingham.



with this consensus sigma sequence. Using these antibodies, we searched for *E. coli* proteins with sigma related proteins (Fujita, N. and Ishihama, A. (1987) *Mol. Gen. Genet.* **210**: 5–9). The specificity of cross-reactive proteins was tested by competition assay using synthetic peptides with contiguous sequences of 5 or 6 amino acids. These synthetic peptides with the consensus sigma sequence and antibodies against these peptides are also useful for identification of the function associated with the consensus sigma sequence.

Strength and Regulation of *Escherichia coli* Promoters, I. Preparation of a Promoter Collection and Determination of Two Parameters of Promoter Strength

Akira ISHIHAMA, Shizuko ENDO*, Hiroji AIBA*, Nobuyuki FUJITA
Rei UESHIMA and Manabu NAKAYAMA

Gene expression in bacterial cells is regulated mainly at the transcription step. The rate of transcription is determined primarily by controlling the rate of initiation. The intrinsic strength of the promoter is, therefore, the primary determinant of the expression level of individual genes in *E. coli* (reviewed in Ishihama, A. (1988) *Trends Genet.*, in press). Up to now, however, measurement and comparison of promoter strength for various genes or operons in *E. coli* has never been attempted due to technical difficulties. Previously, we established an *in vitro* mixed transcription system, in which an equimolar mixture of truncated DNA fragments, each carrying

* Department of Chemistry, Tsukuba University, Tsukuba.

Table 1. Collection of *Escherichia coli* Promoters

Gene	Gene product	Map position	No. of promoters
alaS	Alanyl-tRNA synthetase	58	1
apaH	Diadenosine tetraphosphatase	1	2
asd	Aspartate semialdehyde dehydrogenase	76	2
cca	tRNA nucleotidely transferase (CCA enzyme)	67	—
crp	cAMP receptor protein (CRP)	74	1
cya	Adenylate cyclase	85	1
dgk	Diglyceride kinase	92	1
divE	Cell division control (Serine tRNA)	22	1
did	Respiratory D-lactate dehydrogenase	80	—
dnaQ	DNA polymerase III ε subunit	5	2
frdA	Fumarate reductase	94	1
gal	Galactose fermentation	17	2
glnS	Glutaminyl-tRNA synthetase	16	1
glnV	Glutamine tRNA (a suppressor tRNA)	16	1
gltA	Citrate synthase	17	—
gnd	Gluconate-6-phosphate dehydrogenase	44	1
gor	Glutathione reductase	77	—
groE	GroE ATPase (a heat-shock protein)	94	3
guaB	IMP dehydrogenase	54	1
hupA	HU-2 protein		1
hupB	HU-1 protein	9	2
katG	Catalase HP1	89	—
kdpABC	High-affinity-potassium transport system	16	1
ksgA	16S rRNA methyltransferase	1	1
lac	Lactose fermentation	8	2
leuX	Leucine tRNA (a suppressor tRNA)	97	1
lpp	Murein lipoprotein	36	1
lysT	Lysine tRNA (a suppressor tRNA)	17	1
malB	Maltose fermentation	92	3
nar	Nitrate reductase	27	1
ndh	Respiratory NADH dehydrogenase	22	—
nusA	NusA protein	69	1
plsB	Glycerol-3-phosphate acyltransferase	92	1
ppc	Phosphoenolpyruvate carboxylase	89	1
pyrF	Orotidine-5′-phosphate (OMP) decarboxylase	28	1
recA	RecA protein (Recombination)	58	1
rnh	Ribonuclease H	5	1
rplJ	Ribosomal protein L10	90	1
rpoD	RNA polymerase sigma-70 subunit	67	4
rpoH	RNA polymerase sigma-32 subunit		3

Table 1. (Continued)

Gene	Gene product	Map position	No. of promoters
rpsA	Ribosomal protein S1	21	5
rrnE	ribosomal RNA (operon E)	90	2
serB	Phosphoserine phosphatase	100	2
ssb	Single-strand DNA-binding protein (SSB)	92	1
ssp	Stringent starvation protein (SSP)	70	1
tac	Synthetic promoter (*trp-lacUV5*)	—	1
trp	Tryptophan synthesis	28	1
trxA	Thioredoxin	85	1
tufB	Elongation factor Tu	90	1
unc	Protein-translocating ATP synthase	84	3

a specific promoter and each producing a specific transcript, is transcribed by a known amount of purified RNA polymerase holoenzyme. DNA truncation is designed to produce transcripts with different chain length, which can be separated by electrophoresis on polyacrylamide gels. Using this system, two parameters of promoter strength, the affinity to RNA polymerase and the rate of open complex formation, have been determined for more than 30 promoters (reviewed in Ishihama, A. *et al.* (1987) *RNA Polymerase and the Regulation of Transcription*).

This year, we added more than 50 promoters to our collection of *E. coli* promoters, as listed in Table 1. Preparation of truncated DNA fragments is being carried out in collaboration with Aiba and his colleagues at Tsukuba University. Determination of the promoter strength is in progress in this laboratory.

Strength and Regulation of *Escherichia coli* Promoters, II. Effect of DNA Supercoiling and Temperature Shift on Promoter Activity of the *rpoH* Gene Encoding the Heat-shock Sigma Subunit of RNA Polymerase

Rei UESHIMA, Nobuyuki FUJITA and Akira ISHIHAMA

The *rpoH* gene of *E. coli* codes for the heat-shock sigma subunit (σ^{32}) of RNA polymerase. Heat-shock genes are transcribed only by RNA polymerase holoenzyme containing σ^{32} (Fujita, N. and Ishihama, A. (1987) *J. Biol. Chem.* **262**: 1855–1859). Upon exposure to heat-shock, the in-

tracellular level of σ^{32} increases rapidly. Previously, we found that the induction of σ^{32} synthesis is attributed to the enhancement of *rpoH* gene transcription (Fujita, N. and Ishihama, A. (1987) *Mol. Gen. Genet.* **210**: 10–15). As an attempt to elucidate the enhancing mechanism of *rpoH* gene transcription, the effects of DNA supercoiling and temperature-shift on the activity of two major promoters of the *rpoH* gene were examined using an *in vitro* transcription system. Upstream promoter P1 was not affected by DNA supercoiling nor temperature upshift but downstream promoter P2 was enhanced by both of these factors. Both factors facilitated the formation of an open complex on *rpoH* P2, and these two effects were not additive. Hence we assumed that these two factors act at the same step of transcription, probably the local unwinding of DNA double-helix at the promoter region. Different sensitivities of the *rpoH* major promoters to DNA supercoiling and temperature shift indicate that these promoters are under differential control. Taken together with the heat-shock enhancement *in vivo* of *rpoH* P2 transcription (Fujita, N. and Ishihama, A. (1987) *Mol. Gen. Genet.* **210**: 10–15), we conclude that temperature up-shift itself is a major factor controlling the activity of the *rpoH* promoter under heat-shock stress.

Structure and Function of Genes for RNA Polymerase-Binding Proteins: Regulation of Expression of Stringent Starvation Protein (SSP) Gene

Hiroaki SERIZAWA and Ryuji FUKUDA

A variety of *Escherichia coli* proteins interact with RNA polymerase and may participate in modulating enzyme functions. To identify such transcription factors, we isolated several RNA polymerase-binding proteins and have been studying their effects on transcription in an *in vitro* system. In parallel, we have been trying to isolate the genes coding for these proteins, and to perform genetic analyses of their physiological functions. Stringent starvation protein (SSP) is one of the putative transcription factors. We isolated the gene for this protein (Fukuda, R. *et al.* (1985) *Mol. Gen. Genet.* **201**: 151–157), determined the nucleotide sequence of the gene (Serizawa, H. and Fukuda, R. (1987) *Nucleic Acids Research*, 15, 1153–1163), and identified its genetic map position on the *E. coli* chromosome (Fukuda, R. *et al.* (1988) *Mol. Gen. Genet.* **211**: 515–519).

This year, we continued research on the regulation of expression of the SSP gene. As reported previously, the consensus promoter sequences are not present at the −10 and −35 regions upstream from the transcription initiation site. Deletion mapping studies of the 5′ flanking region, however, indicated that the 36 bp sequence upstream from the transcription initiation site was essential for the promoter function. In addition, it was found that an approximately 40 bp sequence further upstream from this essential promoter region stimulated *in vivo* transcription several fold. This stimulatory effect could not be observed in the *in vitro* transcription system using purified RNA polymerase, and either linear or superhelical template DNA containing this *cis*-acting stimulatory region. The fact suggests that a transcription factor(s) interacts with this region to enhance transcription. DNA fragments containing this *cis*-acting region exhibited a bending structure, as analyzed by gel electrophoresis. The bending center was found to be located around 50 bp upstream from the transcription initiation site.

Analysis of the 5′ flanking sequence showed the presence of two genes (*rplM* and *rpsI*) for ribosomal proteins, L13 and S9. The transcription termination signal for these genes was located around 200 bp upstream from the transcription initiation site of the SSP gene. Detailed S1 nuclease mapping analysis of *in vivo* RNA indicated transcription read-through from these ribosomal protein genes to the SSP gene. Furthermore, transcription in the opposite direction was observed, initiating from the 3′ flanking region of the SSP gene. The meaning of these transcription is not understood yet.

RNA Polymerase and Transcriptional Signals of *Micrococcus luteus*

Manabu NAKAYAMA, Nobuyuki FUJITA, Syozo OSAWA* and Akira ISHIHAMA

Among bacterial species, the G+C content of genomic DNA varies in a wide range from about 25% to 74%. The G+C content of *Micrococcus luteus* DNA is 74%, which is much higher than that of *E. coli* (about 50%). We showed that the codon usage pattern of *M. luteus* is much different from that of *E. coli*, and extremely biased to the use of G and C in silent

* Faculty of Science, Nagoya University, Nagoya.

positions (Ohama, T. *et al.* (1987) *J. Bacteriol.* **169**: 4770–4777).

In order to understand the influence of GC pressure on the structure of transcription signals, particularly the promoter structure, we initiated analyses of promoter structures of *M. luteus* and of the structure and function of RNA polymerase, which recognizes these promoters. By mapping with reverse transcriptase, we determined the initiation site for both *in vitro* and *in vivo* transcription of the *M. luteus* streptomycin (*str*) operon. In an *in vitro* run-off transcription assay using truncated DNA templates, RNA polymerases from both *M. luteus* and *E. coli* were able to transcribe *in vitro* correctly from this *str* promoter. In contrast, the enzyme of *M. luteus* is unable to transcribe correctly from *E. coli* promoters. Thus, we reached the conclusion that the promoter sequences of *M. luteus* are similar to but distinct from those of *E. coli*, and that RNA polymerase of *M. luteus* is stricter in its promoter selection.

Processing of tRNA precursors in *Escherichia coli*: RNase P is involved in both 5′ and 3′ termini of *leuX* tRNA precursor

Teruaki NOMURA and Akira ISHIHAMA

Primary transcripts of stable RNA genes such as rRNA and tRNA genes are biologically inactive precursor molecules. Several enzymes are involved in the processing of these precursors: processing of 5′-termini is catalyzed by RNase P, which is composed of two components, C5 protein and M1 RNA, while processing of 3′ termini is catalyzed by a combination of at least two enzymes other than RNase P.

The *leuX* gene of *Escherichia coli* codes for a suppressor tRNA, which inserts leucine at the amber codon. We found that this gene is transcribed as a monomeric precursor carrying about 20 and 34 base extra sequences at its 5′ and 3′ ends, respectively (Nomura, T. and Ishihama, A. (1987) *J. Mol. Biol.* **197**: 659–670). In spite of the general belief that precursors of stable RNAs are rapidly processed and cannot be detected in wild-type *E. coli* cells with respect to tRNA processing enzymes, we identified the accumulation of primary transcripts of the *leuX* gene. Following measurements of both precursor and mature *leuX* tRNA levels under various growth conditions, we proposed a model in which the expression of *leuX* gene is controlled not only at transcription but also at the tRNA processing step (Nomura, T. and Ishihama, A. (1987) *J. Mol. Biol.* **197**: 659–670).

An analysis of the processing *in vitro* of the *leuX* precursor revealed that the processing of the 5′ end takes place in a single step reaction catalyzed by RNase P while the 3′ processing involved a two step reaction. The activity of initial endonucleolytic cleavage of the 3′ precursor sequence was found to copurify along RNase P. Heat inactivation of RNase P from two independent RNase P mutants abolished the cleavage activity of both the 5′ and 3′ ends. These results suggest that RNase P is involved in not only 5′ processing but also in the initial step of 3′ processing.

In the presence of Mg^{2+} alone, the *leuX* precursor was found to be self-cleaved at a site of about 13 nucleotides towards the inside from the 5′ end of mature tRNA. Self-cleaved precursor tRNA was not addititionally processed by the 3′ endonuclease, suggesting that 3′ endonuclease recognizes a specific conformation of precursor tRNA for action.

Molecular Mechanism of Influenza Virus RNA Transcription, I. Role of NP on RNA Chain Elongation

Ayae HONDA*, Kenji UEDA**, Kyosuke NAGATA and Akira ISHIHAMA

The influenza virus genome consists of eight single-stranded RNA segments of negative polarity. Three P proteins (PB1, PB2 and PA) and one nucleocapsid protein (NP) are associated with each RNA segment, together forming ribonucleoprotein (RNP) cores. After virus infection, the RNP cores are transported into cell nuclei and primary transcripts are produced by the core-associated RNA polymerase (reviewed in Ishihama, A. and Nagata, K. (1988) *CRC Crit. Rev. Biochem.* **23**: 27–76). Biochemical and genetic studies have revealed that the catalytic site for RNA synthesis is located on the P protein complex. The role of NP on the transcription of viral RNA genome is, however, not understood yet.

In order to assign a role(s) to NP protein on transcription, we designed two kinds of dissociation and reconstitution experiments. RNP cores were dissociated into RNA polymerase-RNA genome (P-RNA) complexes and NP protein by CsCl centrifugation. The P-RNA complexes were capable of catalyzing the endonucleolytic cleavage of capped RNA, the

* Present address: Bristol-Meyers Research Institute, Tokyo.
** Present address: Department of Neurosciences, School of Medicine, University of California, San Diego.

initiation of primer-dependent RNA synthesis, and the synthesis of small-sized RNA, but were unable to synthesize full-sized RNA. By adding the isolated NP protein to the P-RNA complexes, RNP complexes were reconstituted, which supported the synthesis of full-sized transcripts as did native RNP cores. These results suggest that NP protein is required for efficient elongation of RNA chains but not for initiation of RNA synthesis.

RNP was then dissociated into RNA-free proteins and a protein-free genome RNA by CsTFA/glycerol centrifugation (Kato, A. *et al.* (1986) *Virus Res.* **3**: 115–127; Honda, A. *et al.* (1987) *J. Biochem.* **102**: 1241–1249). By mixing these two fractions, primer-dependent RNA-synthesizing activity was regained. These complexes, however, produced only small-sized RNA, presumably due to incorrect assembly of NP on viral RNA. To confirm these observations, we developed a transfection assay system of RNP complexes, instead of infection of native virions. Infectivity of the reconstituted RNA-protein complexes is being tested.

Molecular Mechanism of Influenza Virus RNA Transcription, II. Inhibition of Transcription by Matrix Protein

Raleigh W. HANKINS, Kyosuke NAGATA and Akira ISHIHAMA

Until recently, the influenza virus matrix (M)-protein had been thought to function solely as a template for the attachment of both surface proteins and nucleocapsid cores during the course of virus maturation. Within the past few years, however, a number of reports have been published which implicate M protein in the inhibition of viral RNA (vRNA) transcription. While the involvement of M protein in such a role is well supported by experimental evidence, its exact nature has not been characterized adequately. It has been the purpose of our research to elucidate the mechanism by which influenza virus M protein effects vRNA transcription inhibition. Specifically, we set out to clarify the following aspects of this M protein function: 1) the stage of vRNA transcription which M protein inhibits, *i.e.*, initiation versus elongation, 2) the portion of the basic viral transcription unit (RNP) to which M protein binds to effect inhibition; 3) whether this binding occurs in a specific manner.

The results of our experiments enabled us to obtain a much better pirture on how influenza virus M protein affects vRNA transcription inhibition. Analyses of vRNA transcription products in the presence of varying amounts

of M protein, and under differing substrate conditions led us to conclude that M protein-effected inhibition is exerted mainly at the level of vRNA transcription initiation and/or reinitiation. When nucleocapsid protein (NP) was removed from RNP, the addition of M protein to the remaining RNA polymerase-RNA (P-RNA) complexes had no effect on vRNA transcription. Thus, inhibition itself probably occurs when M protein binds to NP and physically blocks movement of the viral RNA polymerase along vRNA. Finally, the observation that only certain monoclonal anti-M antibodies were effective in neutralizing M protein, independent of their Western blot titers, strongly suggests that M protein-affected vRNA transcription inhibition occurs by site-specific binding of M protein to NP.

Presently, we are in the process of utilizing a battery of anti-M monoclonal antibodies in developing an M protein epitope map of the functional site(s) involved in vRNA transcription inhibition.

Transcription and Replication of Influenza Virus RNA: An *in vitro* System Using Isolated Nuclei from Virus-Infected Cells

Kyosuke NAGATA and Akira ISHIHAMA

We developed an *in vitro* system for transcription and replication of influenza virus RNA using isolated nuclei from influenza virus-infected HeLa cells (Takeuchi, K. *et al.* (1987) *J. Biochem.* **101**: 837–845). In this system, two species of positive-sense RNA tanscripts (mRNA and cRNA) and one negative-sense RNA transcript (vRNA) are synthesized.

In the course of fractionation of this nuclear system by centrifugation, we found that the precipitates of nuclear extracts (NEP) predominantly produced mRNA while the supernatant (NES) alone exhibited a low level of cRNA synthesis activity. With the addition of the NES fraction, mRNA synthesis by NEP was switched to cRNA synthesis. This activity was not detected in nuclear extracts prepared from unifected cells and was heat-labile. Glycerol density centrifugation of the NES fraction in the presence of high salt, however, yielded RNA polymerase complexes that supported mRNA synthesis. These observations suggest that a regulatory factor(s) either encoded by the virus genome or induced by virus infection is involved in the switch from mRNA to cRNA synthesis, and that this putative factor(s) can be dissociated from RNA polymerase complexes upon exposure to

high ionic strength. Isolation and characterization of such a factor(s) are in progess.

Translational Regulation of Influenza Virus mRNAs

Kunitoshi YAMANAKA, Kyosuke NAGATA and Akira ISHIHAMA

In influenza virus-infected cells, ten kinds of viral proteins are synthesized and their synthesis is regulated with respect to total amount and timing. Recent studies revealed that transcription is the key step for gene expression of influenza virus (reviewed in Ishihama, A. and Nagata, K. (1988) *CRC Crit. Rev. Biochem.* **23**: 27–76). The contribution of posttranscriptional control is, however, not yet known.

To elucidate translational regulation in influenza viral gene expression, we inserted cDNA sequences containing ATG for the initiation codon of translation of influenza viral mRNAs into the cap-proximal leader region of the CAT gene in a vector, pSV2cat. Two plasmids, designated pSV2cat and pSV2NS1cat, were constructed using cDNAs for segments 6 and 8 conding for NA and NS proteins, respectively. Both plasmids lead to the synthesis of complete CAT protein fused to N terminal portions of NA and NS proteins. HeLa cells were transfected with either pSV2NA1cat or pSV2NS1cat and, after 24 hr, superinfected with influenza virus. CAT activity in cells transfected with pSV2NS1cat reached a maximum during the early stage of infection, whereas maximal CAT activity in cells transfected with pSV2NA1cat was obtained during the late stage of infection. These variations in patterns of CAT activity are consistent with the known order of gene expression of NS and NA proteins. The primer-extension analysis indicated that intracellular distribution and metabolic stability of mRNA encoding fused CAT protein were similar between cells transfected with pSV2NS1cat and pSV2NA1cat. From these results, we propose that translational control operates during influenza virus infection and that the signal involved in this control is located around the translation initiation site.

Establishment of Mouse Cells Expressing Influenza Viral Genes

Kunitoshi YAMANAKA, Kyosuke NAGATA, Yasuhiro HOSAKA*
and Akira ISHIHAMA

Recent studies have revealed that cellular immunity plays a crucial role in protection against influenza virus infection. In order to identify protein(s) and epitope(s), which are recognized by cytotoxic T lymphocytes, we attempted to establish mouse cells producing individual influenza viral proteins. Along this line, we focused on nucleocapsid protein (NP) because NP from various subtypes of influenza virus share a similar antigenicity and NP-reactive cytotoxic T lyphocytes are therefore expected to show cross-reactivity against various subtype viruses.

For efficient expression of cDNA for NP protein in mouse cells, we inserted NP cDNA under control of mouse metalothionein I promoter into a bovine papiloma virus-based vector containing a neomycin resistant gene. The recombinant plasmid was transfected into mouse L cells by the calcium-phosphate coprecipitation method and Neo-resistant colonies were selected with G-418. Expression of the NP protein gene was tested by Western blotting analysis using anti-NP antibody. Cells expressing the NP protein gene were tested with respect to reaction against cytotoxic T lymphocytes. The NP-expressing mouse cells were also were used for transfection of RNA polymerase-viral RNA (P-RNA) complexes devoid of NP (see Honda, A. *et al.* in this report).

In order to determine the roles of viral proteins in transcription and replication, attempts have been made to establish cells producing P proteins and non-structural (NS) proteins. These cells are presently beines used to examine self-replication capabilities of protein-free RNA, RNA polymerase-viral RNA (P-RNA) complexes and RNA polymerase-NP-viral RNA complexes (RNP).

* Research Institute of Microbial Diseases, Osaka University, Suita.

Control of Influenza Virus Gene Expression: Quantitative Analysis of Each Viral RNA Species in Infected Cells

Eriko HATADA*, Masakazu HASEGAWA**, Jun MUKAIGAWA***, Kazufumi SHIMIZU*** and Ryuji FUKUDA

For studies on control mechanisms of RNA synthesis in influenza virus-infected cells, it is necessary to develop a simple system for measuring the quantity of each viral RNA species. As reported last year, we established a quantitative hybridization system, in which the quantities of three types of influenza virus RNAs (vRNA, mRNA and cRNA) could be measured separately for the 8 genome segments (24 RNA species in all). As the hybridization probes in this system, ^{32}P-labeled RNAs of both plus and minus polarity were synthesized employing a SP-6 transcription system. For assay, these probes were used in a large molar excess sufficient to overcome complementary RNAs present in RNA samples from infected cells.

Employing this hybridization system, we first analyzed the time course of the accumulation of each viral RNA species in MDCK cells infected with A/Udorn/72 (H3N2) and compared it with the time course of the viral protein synthesis. During the early phase of infection (1 h postinfection), appreciable levels of mRNA appeared for segments 1, 2, 3, 5 and 8 (encoding the early proteins). This pattern of mRNA synthesis was similar to that observed in virus-infected cells in the presence of inhibitors of protein synthesis, such as cycloheximide. The result indicates that a differential control operates at the transcriptional level during the primary transcription. At 2 h postinfection, the synthesis of vRNA as well as cRNA began simultaneously for all segments. The vRNA level continued to increase beyond 5 h postinfection, while the cRNA level reached a plateau at 2 h postinfection. mRNAs encoding the late proteins started to be synthesized in close coupling with the rise of vRNAs, and thereafter the level of both early and late mRNAs, except mRNAs for polymerase proteins, continued to increase with the rise of the respective vRNAs. The mRNA levels did not increase but rather declined after 3 h postinfection for all segments, being completely uncoupled with vRNA levels. We could not detect any

* Present address: School of Medicine, Nihon University, Tokyo.
** Present address: Chugai Exploratory Research Labs, Gotemba.
*** School of Medicine, Nihon University, Tokyo.

delay in the synthesis of vRNA segments encoding the late genes. This observation does not agree with the previous concept on the control mechanism of viral RNA synthesis.

The accumulation pattern of mRNAs encoding three polymerase proteins was rather exceptional. The synthesis of mRNA for P proteins ceased after 2 h postinfection, and the mRNA level remained at a low level thereafter. The synthesis rate of NP protein increased in proportion to the level of its mRNA, while that of the M1 protein remained at a low level during the early phase in the presence of a high level of mRNA. It increased abruptly thereafter during the late phase of infection. Results thus indicates that the gene expression of influenza virus is regulated not only at the level of transcription, but also at some posttranscriptional step(s).

Studies on the Function(s) of Influenza Virus Nonstructural Proteins

Eriko HATADA*, Masakazu HASEGAWA**, Kazufumi SHIMIZU***
Masakazu HATANAKA**** and Ryuji FUKUDA

To study the function(s) of nonstructural protein (NS1) of influenza virus, we analyzed temperature-sensitive mutants of influenza virus A/Udorn/72 (H3N2) having lesions in RNA segment 8. As reported last year, the nucleotide sequence of segment 8 was determined for three of these mutants. Both ICRC1629 and SPC45 have mutations in the NS1-coding region, whereas ICRC516 has a mutation in the NS2-coding region. The synthesis of virus-specific proteins was analyzed in MDCK cells infected with these mutant viruses. With both NS1 *ts* mutants, the syntheses of two late proteins, M1 and HA, were greatly reduced at a nonpermissive temperature of 40°C compared to 34°C. The synthesis of NS1 also decreased at the nonpermissive temperature. On the other hand, no significant alteration was observed with the NS2 *ts* mutant in the synthesis of viral proteins at 40°C.

Using a quantitative hybridization method as described above, the syn-

* Present address: School of Medicine, Nihon University, Tokyo.
** Present address: Chugai Exploratory Research Labs, Gotemba.
*** School of Medicine, Nihon University, Tokyo.
**** Institute for Virus Research, Kyoto University, Kyoto.

thesis of virus-specific RNAs was analyzed. The synthesis of viral mRNAs was retarded, reflecting the delayed and reduced synthesis of vRNAs, with all three mutant viruses. For both NS1 mutants, the levels of individual mRNAs synthesized at 40°C were almost the same as those at 34°C, although severe reduction was observed at 40°C in the synthesis of the late proteins and NS1. These observations suggest that the NS1 protein is involved in at least two distinct steps of the virus growth process: one in the synthesis of vRNA and/or cRNA; and another in the posttranscriptional process(es) of late protein synthesis.

Analysis of a Novel Influenza A Virus PB2 Mutant, Defective in the Regulation of Three Polymerase Genes

Jun MUKAIGAWA*, Eriko HATADA**, Ryuji FUKUDA and Kazufumi SHIMIZU*

To reveal the function(s) of the PB2 protein of influenza A virus, six temperature-sensitive (*ts*) mutants of A/Udorn/72 (H3N2) virus, each having a *ts* mutation in the PB2 gene, were analyzed for the synthesis of viral RNAs and proteins. One of the mutants, ICRC27, exhibited novel phenotypes, and was characterized in detail. At a nonpermissive temperature of 40°C, the synthesis of complementary RNA (cRNA; template for genome RNA synthesis) for each genome segment was blocked, resulting in a severe reduction in the synthesis of genome RNA. As a result, the secondary transcription was also blocked. Accordingly, viral late proteins (M and HA) were barely detectable and the syntheses of NP and NS1 proteins decreased and/or was delayed.

At a permissive temperature of 34°C, the syntheses of both mRNAs and cRNAs of PB2, PB1 and PA segments, which stop at 2 to 3 h postinfection with the wild-type virus, continued at least up to 7 h post infection, yielding several times more copies than did the wild-type virus. Elevated levels of these mRNAs led to increased synthesis of the polymerase proteins. The ts^+ revertants of ICRC27 exhibited none of these mutant phenotypes. These observations indicate that this mutant is defective in the regulatory mechanism(s) underlying the synthesis of mRNAs and/or cRNAs of the

* School of Medicine, Nihon University, Tokyo.
** Present address: School of Medicine, Nihon University, Tokyo.

three polymerase genes, and that the PB2 protein participates in this regulation.

Assignment of the Function of Nuclear Factor I

Ken MATSUMOTO*, Kyosuke NAGATA and Fumio HANAOKA*

Nuclear factor I (NFI) has been isolated from uninfected HeLa cell nuclei as an essential factor for the initiation of adenovirus (Ad) DNA replication, and shown to be a sequence-specific DNA-binding protein. NFI-binding sites, (T/G)GGN$_{6\sim7}$GCCAA, have been identified for a number of cellular and viral genes. Although these sites are located within regulatory regions for transcription, their roles have not been determined yet. Recently, it has been proposed that NFI is identical with one of the CCAAT box-binding proteins (CTF), which has been characterized as a transcription factor.

To test this possibility, we employed a gel retardation assay using a ^{32}P-labeled Ad DNA probe containing the NFI site. Formation of NFI-DNA probe complexes was completely prevented by non-labeled oligonucleotides containing a NFI-binding sequence. In contrast, oligonucleotides containing the CCAAT box did not exhibit efficient competition. DNase I protection analysis revealed that highly purified NFI was unable to bind such CCAAT boxes as those present in promoter regions of the HSV-tk gene and mouse β-globin gene. These results suggest that NFI is a distinct protein from CTF or that NFI binds to CCAAT boxes with very low affinity by itself.

Nuclear extracts prepared from MDCK cells (canine) contained a high level of NFI DNA-binding activity compared to those from HeLa cells (human) and MF3A cells (mouse). Among mouse tissues, nuclear extracts from liver, kidney and spleen exhibited higher NFI DNA-binding activity than those from lung, brain and heart. Furthermore, in both MDCK and mouse kidney nuclear extracts, we detected a novel DNA-binding protein, designated nuclear factor K (NFK), that bound to a sequence adjacent to the NFI site in Ad DNA. Since the NFK binding site in Ad DNA contains a sequence similar to the octamer motif, NFK may be a member of the octamer binding proteins. NFK was capable of binding to this sequence

* Faculty of Parmaceutical Sciences, University of Tokyo, Tokyo.

only in the absence of NFI. These findings suggest that NFK plays a role in kidney-specific gene expression. Studies on possible involvement of NFK in Ad DNA replication, and of both NFI and NFK in transcription are in progress.

GTP Induces Knotting, Catenation, and Relaxation of DNA with Stoichiometric Amounts of DNA Topoisomerase II from *Bombyx mori* and HeLa Cells

Susumu HIROSE, Hisahiro TABUCHI and Koichi YOSHINAGA*

Most of the biochemical properties of DNA topoisomerase II have been characterized under catalytic conditions in which the molar ratio of enzyme to DNA is far below unity. This molar ratio does not seem to reflect an intracellular state where DNA is attached to the chromosome scaffold or nuclear matrix at a 5-90-kilobase distance possibly through topoisomerase II. This led us to investigate the reaction of topoisomerase II under stoichiometric conditions. Type II DNA topoisomerases isolated from the posterior silk glands of *Bombyx mori* and HeLa cells utilize ATP for unknotting of knotted DNA, relaxation of supercoiled DNA, and catenation/decatenation of circular duplex DNA under catalytic conditions. In these reactions, ATP cannot be replaced by GTP. However, GTP induces knotting, catenation, relaxation, but not decatenation of circular duplex DNA with stoichiometric amounts of these enzymes. Only a limited round of the reactions proceed with a concominant hydrolysis of GTP and then the reaction pauses. These GTP-dependent reactions may be employed for maintenance of the knotted an/or catenated state they formed. For details, see *J. Biol. Chem.*, **263**, 3805–3810.

In Vitro Transcription of Eukaryotic Genes Is Affected Differently by the Degree of DNA Supercoiling

Susumu HIROSE and Yoshiaki SUZUKI**

We examined whether topological tension of DNA is required for the regulation of gene expression in eukaryotes. In a posterior silk gland ex-

* Department of Chemistry, Faculty of Science, Shizuoka University, Shizuoka.
** Department of Developmental Biology, National Institute for Basic Biology, Okazaki.

tract, covalently closed circular (ccc) DNA is in a superhelical state that supports more transcription of the fibroin gene than does linear DNA. A HeLa cell extract showed neither a supercoiling activity nor preference for the transcription of ccc DNA over linear DNA. These activities could be added to the HeLa cell extract. Phosphocellulose fractionation of the posterior silk gland extract yielded a flow-through fraction and a 0.6 M KCl eluate fraction which were required for the supercoiling and for efficient transcription of the ccc template in the acceptor HeLa cell extract. The 0.6 M KCl fraction had DNA topoisomerase II activity, and the flow-through fraction contained a supercoiling factor which, with the aid of topoisomerase II, introduced negative supercoils into ccc DNA. When both fractions were added to the posterior silk gland extract, more supercoiling occurred than with the extract alone. Several genes were optimally transcribed under various degrees of supercoiling. The fibroin gene and adenovirus 2 major late promoter were fully transcribed as partially supercoiled templates. The sericin gene required more supercoiling for full transcription, whereas no preference for supercoiling was seen with the transcription of hsp70. These results suggest that DNA topology plays a role in the regulation of gene expression. For details, see *Proc. Natl. Acad. Sci. USA*, **85**, 718–722.

Changes in Organization of Mitochondrial and Nuclear DNA in Cytoplasmic Reversion of Male Sterile Rice

Saburo NAWA, Masa-Aki YAMADA and Yoshio SANO

Strains of rice which exhibit cytoplasmic male sterility (*cms*) contained the two free plasmids, B-1 and B-2, not only in the mitochondrial fraction, but also in the nuclear fraction. These DNAs were no longer apparent in preparations of either mitochondrial or nuclear DNA from cytoplasmically reverted strains which were generated by the treatment of a *cms* strain with EMS. Hybridization with restriction fragments revealed the presence of various sequences homologous to B-1 and B-2 in the mitochondrial genomic DNA of high molecular weight and also in the nuclear chromosomal DNA, both in *cms* strains and in the normal strain. The reversion of male sterility to the fertile conditions was accompanied by the disappearance of the free plasmid DNAs, B-1 and B-2, and the appearance of new patterns of restriction fragments of mitochondrial or nuclear DNA with respect to sequences

homologous to B-1 or B-2. However, no evidence was obtained to support the possible integration of B-1 or B-2 into mitochondrial or nuclear DNA during the reversion. Hybridization revealed several regions of homology, in both mitochondrial and nuclear DNA, common to the sterile strains and to the fertile revertants, but several homologies observed in the *cms* strains were no longer present in the fertile revertants. Thus, *cms* strains contained more homologous sequences in terms of both number and quantity of homologous regions than the normal strain or revertants.

The presence of sequences homologous to B-1 and B-2 in the mitochondrial DNA of the normal strain may mean that plasmid DNAs could have arisen from the mitochondrial genomes by excision, and have then been autonomously replicated and subsequently integrated into various sites on the mitochondrial genome, as transposable elements. These specific events might be associated with the origin of cytoplasmic male sterility in rice. Recombinations or rearrangements of the mitochondrial genome during the reversion might be the cause of the different restriction patterns of DNA from *cms* strains and from revertants. Hybridization data revealed the presence of common DNA sequences in nuclear and mitochondrial genomes in rice. The episomal plasmids, B-1 and B-2, may have left the mitochondria of *cms* strains and entered the nucleus where they were transposed into the genome. The B-1 and B-2 sequences in nuclear DNA may perhaps be able to serve as vectors of the free plasmids for transformation in rice, since it may be possible to integrate foreign DNA into the genome by transposition or recombination of the homologous sequences.

The presence of the free plasmids and the characteristics of the organization of the mitochondrial and nuclear genomes did not affect the ability of the plant to produce functional pollen when the nuclear background contained nuclear restorer genes. However, both the disappearance of the free plasmids and the rearrangements in mitochondrial DNA of high molecular weight or in nuclear DNA, with respect to B-1 and B-2 sequences, were involved in the reversion to fertility, and these events are indicative of some correlation between the organization of these sequences and cytoplasmic male sterility in rice.

Messenger RNA Synthesis of Sendai Virus (HVJ)

Kiyohisa MIZUMOTO

Sendai virus (HVJ), a member of the paramyxovirus group, contains a single nonsegmented RNA genome of approximately 15 kb long with negative polarity encoding at least seven proteins, NP, P, C, M, F, HN and L. The genetic information of this virus appears to be expressed through six monocistronic mRNAs corresponding to each protein, except that the P and C proteins are translated from a single mRNA species with two overlapping reading frames. It has been suggested that these mRNAs are synthesized by transcribing the negative strand genome RNA with the virion associated RNA-dependent RNA polymerase, which is assumed to consist of the P and L proteins. The precise mechanism of the transcription, however, is unclear, because no efficient *in vitro* transcription system has been established. We have tried to develop a transcription system in which faithful initiation as well as termination takes place.

We have found that the *in vitro* transcription directed by purified HVJ particles is almost entirely dependent on the addition of host (HeLa) cell extracts. The active component (transcription factor) in the extracts was heat labile and sensitive to trypsin-treatment, while refractory to nuclease-treatment. The transcription factor activity was also detected in other animal cells, and even in plant cells, indicating that HVJ utilizes protein(s) ubiquitously present in the eukaryotic cells for its transcription. The transcription factor was partially purified from bovine brain and was shown to be separated into at least two complementary fractions, one of which could be replaced by highly purified tubulin.

The *in vitro* reaction products largely consisted of 18S and 12S RNA species which were similar in size to the mRNAs made in the infected cells. A single cap structure, $m^7GpppAm$, was released from *in vitro* poly(A)$^+$ RNA upon digestion with nuclease P1, indicating that transcription of all the HVJ mRNA starts with the A residues. Identical 5′-cap structures were found on the viral mRNAs made *in vivo*. From RNA-RNA hybridization and protection experiments with minus strand genome RNA, the *in vitro* products were predominantly of message sense (plus strand), and were transcribed from correct sites on the genome.

Mechanism of mRNA Capping Reaction

Kiyohisa MIZUMOTO

Most of the cellular as well as viral mRNAs in eukaryotes contain a 5′-terminal cap structure, m^7GpppN. The cap structure is required for the efficient initiation of translation and for RNA splicing. It has also been suggested that the synthesis of the methylated cap structure and/or the association of the capping enzyme system with RNA polymerase may play an important role in the initiation of transcription. The cap structure is synthesized at the initial stage of mRNA synthesis and is conserved at the 5′-termini of RNAs while they are processed in the nucleus and transported to the cytoplasm. Consequently, elucidation of the mechanism of synthesis of the cap structure is important for understanding the molecular mechanism of eukaryotic gene expression.

We characterized the reactions catalyzed by the mRNA capping enzyme (mRNA guanylyltransferase) which is a key enzyme in the cap formation, to understand the molecular mechanism of steps involved in eukaryotic mRNA synthesis. Using mRNA capping enzymes from various eukaryotic cells, we demonstrated that the capping reaction (GTP+ppN-RNA→ G(5′)pppN-RNA+PPi) proceeds through a covalent enzyme-GMP intermediate in which GMP is linked to the ε-amino group of a lysine residue throug ah phosphoamide bond (Mizumoto, K. and Kaziro, Y. (1987), *Prog. Nucl. Acid. Res. Mol. Biol.*, **34**, 1–28).

An RNA 5′-triphosphatase activity specifically hydrolyzing the γ-phosphate from the 5′-triphosphate terminated RNA was found to be tightly associated with guanylyltransferase purified from rat liver, *Artemia salina*, and yeast suggesting that this is a general feature of the capping system. The capping enzyme from rat liver and *A. salina* consists of a single polypeptide chain with Mrs of 69,000 and 73,000 respectively, containing catalytic domains for both guanylyltransferase and triphosphatase. In contrast, a highly purified yeast capping enzyme is composed of two separate chains of 52 kDa (α) and 80 kDa (β), responsible for the activities of guanylyltransferase and triphosphatase, respectively (Itoh, N. *et al.* (1987), *J. Biol. Chem.*, **262**, 1989–1959; Mizumoto, K. and Kaziro, Y. (1987), *Prog. Nucl. Acid Res. Mol. Biol.*, **34**, 1–28).

To see whether the α and β subunits are derived from a single polypeptide or are independent polypeptides encoded by two separate genes, we at-

tempted to isolate the gene(s) coding for the yeast capping enzyme. A yeast genomic DNA expression library in λgt11 was screened with an antibody against the yeast capping enzyme which recognizes both α and β chains. One of the positive clones, λC3, contained a 3,500-base pair insert of yeast DNA. From experiments based on the affinity selection of the antibody by antigens produced with λC3 in *E. coli*, this clone appeared to contain the guanylyltransferase gene. The insert contained an open reading frame encoding a polypeptide of 459 amino acids with a calculated Mr of 52,764, corresponding to the size of the 52 kDa α subunit. The identiry of the gene was further confirmed by expressing the gene in *E. coli* to give catalytically active guanylyltransferase.

From a Northern blot analysis of the yeast poly(A)$^+$ RNA, the size of the guanylyltransferase mRNA was estimated to be about 1,600 bases. Since this mRNA size fairly closely corresponds to a 52 kDa polypeptide, the α and β chains of the yeast capping enzyme are probably encoded by two separate genes. The disruption of one copy of the α subunit gene in a diploid yeast creates a recessive lethal mutation, indicating that the single guanylyltransferase gene of yeast has an essential function.

Regulation of Initiation of Chromosomal Replication in Bacteria

Hiroshi YOSHIKAWA

(1) Regulation of initiation of the chromosomal replication by DnaA-boxes in the origin region of the *Bacillus subtilis* chromosome.

A gene homolgous to the *Escherichia coli dnaA* gene and two flanking regulatory regions which contain nine and four DNA-boxes (the sequence of 9 nucleotides present in *oriC* of *E. coli* and serves for the initial binding sites of DnaA protein to activate *oriC*: the consensus sequence is TTATCCACA), respectively are located in the replication origin region of the *B. subtilis* chromosome (Ogasawara et al. 1985 EMBO J. 4: 3345). Attempts to isolate an autonomously replicating fragments from these regulatory regions in order to identify *oriC* have not been successful because the DnaA-box containing regions strongly inhibited plasmid transformation particularly when inserted into a high copy number plasmid pUB110. Using two plasmids differing in copy number, the two regions were subdivided into three regions, A, B and C, each containing 5, 4 and 4 DnaA-

boxes, respectively, which differed in level of inhibition of transformation. Region C is downstream of the *dnaA* gene and inhibits transformation in high-copy but not in low-copy number plasmid. When a part of the DnaA-boxes was deleted from the incompatible plasmids, they became transformable and produced slow-growing transformants in which the initiation frequency of chromosomal replication was selectively reduced. Fast-growing revertants were found containing the same number of plasmids as the parent but with single base changes in the DnaA-boxes. These mutations were in the most highly conserved bases of the DnaA-box sequence. This indicates that a sequence-specific interaction of the DnaA-box most likely with the *B. subtilis* DnaA protein is responsible for the observed incompatibility and thus appears to be involved in control of initiation frequency of the chromosomal replication.

(2) Structure of *dnaA* region of *Pseudomonas putida*: Conservation among the three bacteria, *Bacillus subtilis*, *Escherichia coli* and *P. putida*.

We have cloned from *Pseudomonas putida* a gene homologous to *Escherichia coli dnaA*, and determined the sequence of the gene and its neighboring region. The *dnaA* gene and at least 3 other genes, *dnaN*, *recF* and *gyrA* are found highly homolgous with the genes in the *dnaA* region of *E. coli* and *B. subtilis*. A large non-translatable region immediately upstream of the *dnaA* gene is also conserved in the three bacteria and contains 3, 12 and 14 DnaA-boxes (TTATCCACA and closely related sequences) in *E. coli*, *P. putida* and *B. subtilis*, respectively. The present results confirm our hypothesis that *dnaA* region is the replication origin region of the ancestral bacteria and the essential feature of the *dnaA* and *DnaA-box* combination is conserved in most of the eubacteria to play a central role in initiation of chromosomal replication.

II. MICROBIAL GENETICS

A Synthetic Translation Terminator Gene: a Tool for Dissecting Translation Direction of Gene

Ichiro N. MARUYAMA*, Kazuhiro HORIKOSHI**, Yasukazu NAGASE**
Masaaki SOMA**, Masahiro NOBUHARA**, Seiichi YASUDA
and Yukinori HIROTA†

A 41-nucleotide-long duplex DNA fragment (TER fragment), which contains a TAA translation terminator in all six reading frames joined to the *lac* operator sequence of *Escherichia coli* (Fig. 1), was synthesized. This fragment may be useful not only for producing a truncated protein encoded in a plasmid, but also for the identification of the precise coding region and translation direction of a bacterial gene in the cloned chromosomal segment. By inserting the TER fragment into two sites in the coding region of a gene, one can determine the precise coding region and the translation direction by molecular weight measurement of the two truncated proteins. The *lac* operator system was used for the selection of the plasmid containing the synthetic fragment. We could detect the insertion of the TER fragment into plasmid DNA which has a copy number of more than 20 per cell. The *lac* operator on the plasmid binds with the *lac* repressor which is present at about ten molecules per cell. As a result, the *lac* operon in the host chromosome is expressed.

The TER fragment was inserted into the β-lactamase structural gene in pBR322 in order to test *in vivo* activity. The plasmid produced mutant β-lactamase reduced in size, as expected from the insertion site, and rendered the host bacterium constitutive for β-galactosidase. Thus, termination

† Deceased on December 23, 1986.
Yukinori Hirota left many works unfinished, along with a great legacy of the "temperature-sensitive mutant stock of *Escherichia coli*" as an achievement of his 'K-project'. This and the following seven reports describe the results achieved by collaboration of the late Professor Hirota and the joint authors who worked with him during his lifetime and continued the researches left unfinished after his death.

* Present address: Medical Research Council Molecular Genetics Unit, Cambridge, England.
** Mochida Pharmaceutical Co. Ltd., Tokyo.

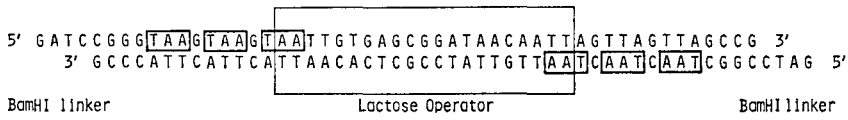


Fig. 1. Nucleotide sequence of the TER fragment.

codons and the lactose operator in the synthetic nucleotide appear to be functional *in vivo*. For details, see *Gene Anal. Tech.*, in press.

Determination of Gene Products and Coding Regions from the *murE-murF* Region of *Escherichia coli*

Ichiro N. MARUYAMA*, Aki-hiko YAMAMOTO* and Yukinori HIROTA†

The *murE* and *murF* genes of *Escherichia coli* encode diaminopimelic acid- and D-alanyl-D-alanine-adding enzymes, respectively, and both are involved in the biosynthesis of the cytoplasmic precursor of cell wall peptidoglycan, UDP-*N*-acetylmuramyl-L-Ala-D-γ-Glu-*meso*-diaminopimelyl-D-Ala-D-Ala (UDP-MurNAc-pentapeptide). Both genes are located at 2 min in the genetic map and are contained in a large cluster of genes for cell envelope growth and division. To understand how peptidoglycan synthesis and septum formation are organized during cell growth in *E. coli*, a structural analysis of this gene cluster is important.

The *murE* and *murF* genes are adjacent to the *ftsI* gene and carried by pLC26-6 (Takeda, Y. *et al.*, 1981, Plasmid **6**: 86). We subcloned DNA segments from pLC26-6 and determined the precise position of *murE* and *murF* genes on the restriction map as summarized in Fig. 1. The product of *murE* and *murF* genes were 56 kDa and 54 kDa proteins, respectively. These genes were transcribed in the same direction as the *ftsI* gene (clockwise on the genetic map). The *murE* gene seems to have its own promoter. It is not clear whether *murF* is organized in an operon together with *murE* or transcribed independently.

† See footnote to p. 39.
* Present address: Medical Research Council Molecular Genetics Unit, Cambridge, England.
** Present address: Department of Biology, Kohnan University, Kobe.

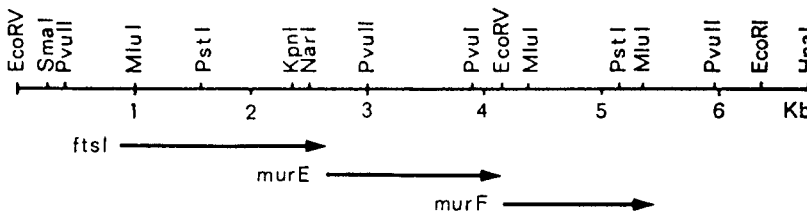


Fig. 1. Restriction map of *ftsI-murF* chromosomal region. Arrows indicate the polypeptide-coding regions and transcriptional directions of the genes.

A Note on the *parA* Mutation in *Escherichia coli*

Jun-ichi KATO*, Hideho SUZUKI, Yukinobu NISHIMURA
and Yukinori HIROTA†

Thermosensitive *par* mutants of *Escherichia coli* have been described as mutants that grow into filamentous cells with centrally located nucleoids at restrictive temperatures and the *parA* mutation responsible for such characteristic morphology was mapped around 95 min close to *purA* (Hirota, Y. *et al.*, 1971, *Biomembranes* **2**: 13). The thermosensitive growth of the *parA* mutant MFT110 was partially corrected by pLC8-47 (Nishimura, Y. *et al.*, 1977, *Plasmid* **1**: 67).

The restriction sites on pLC8-47 were determined for several nucleases as shown in Fig. 1. The *Kpn*I fragment was recloned from the chromosomal region of pLC8-47 and various deletions were introduced (Fig. 1). The recloned constructs were introduced into MFT110 and examined for the ability to correct thermosensitivity in growth. Results are included in Fig. 1 and indicate that the *ca.* 1.6 kb *Bam*HI-*Pvu*II fragment of the chromosome region in pLC8-47 contained a putative gene to complement the thermosensitive mutation in MFT110 and that the *Hpa*I site on this fragment is essential for complementing ability. However, pJK710 carrying the *Bam*HI-*Pvu*II region did not correct the Par phenotype of MFT110, nor did pLC8-47K which was identical with pLC8-47 except that two small contiguous *Pst*I fragments of the plasmid region were replaced by a kanamycin resistance gene (Fig. 1). Clearly, the chromosome region carried by pLC8-47 does not cover the *parA* gene mutated in MFT110. MFT110

† See footnote to p. 39.

* Department of Bacteriology, National Institute of Health, Tokyo.

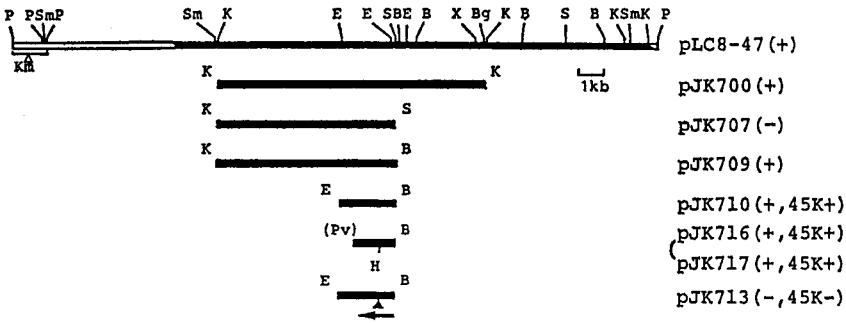


Fig. 1. Analysis of the DNA region that corrects the thermosenstivity of *parA* mutant MFT110.

Solid and hollow bars denote the regions derived from the chromosome and the plasmid, respectively. Only the cloned regions are shown except for pLC8-47. pJK716 and pJK717 had the same *Bam*HI-*Pvu*II chromosome region but with an opposite orientation: in pJK717 the *lac* promoter was joined to the *Bam*HI end and in pJK716 to the *Pvu*II end which was converted to a *Bam*HI cohesive end in joining. A filled triangle indicates the insertion site of a translation terminator and an arrow shows the approximate location and the transcriptional direction of the gene that corrects the thermosensitive defect of MFT110 and codes for a 45K protein. An open triangle with Km indicates the deleted region and the insertion site of a kanamycin resistance gene in pLC8-47K (*cf.* text). Symbols in parentheses following the plasmid numbers denote that complementation of the thermosensitive mutation in MFT110 was positive (+) or negative (−) and that the synthesis of the 45K protein was detected (45K+) or not (45K−). B, *Bam*HI; Bg *Bgl*II; E, *Eco*RI; H, *Hpa*I; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; Sm, *Sma*I; X, *Xho*I.

possibly has at least two thermosensitive mutations and the major thermosensitive defect is not due to *parA*. The *parA* mutation should also give rise to a thermosensitive defect, which might be suppressed phenotypically to some extent under ordinary culture conditions. The major thermosensitive defect in MFT110 may be due to *psd* which is also mapped close to *purA*, because pJK717 as well as pLC8-47 corrected the thermosensitivity of GC2418 (*psd*-1) and YYC176 (*psd*-2).

The gene product directed by the *Bam*HI-*Pvu*II fragment was identified as a 45 kDa (45K) protein by comparing the electrophoretic spectra of *in vitro* products directed by pJK710 and pJK713. The insertion of a translation terminator into the *Hpa*I site on pJK710 to construct pJK713 resulted

in the simultaneous loss of abilities to direct the synthesis of the 45K protein and to correct the thermosensitive defect in MFT110. The synthesis of the 45K protein *in vitro* was more augmented with pJK717 than with pJK716 (*cf.* legend to Fig. 1), and IPTG (isopropyl β-D-thiogalactoside) stimulated the 45K protein synthesis directed by pJK717 in maxicells. The transcription for the 45K protein appears to proceed in the direction from the *Bam*HI to the *Pvu*II site.

The 45K protein may be a *psd* product. The *psd* product, a phosphatidylserine decarboxylase, is an essential enzyme involved in the biosynthesis of phosphatidylethanolamine, the major membrane lipid of *E. coli*. Although it is possible that *psd* may affect chromosome partition, the present results rather indicate that the *parA* locus is located somewhere other than at the 95 min position.

A New Mutation Causing a Defect in Chromosome Partition in *Escherichia coli*

Yukinobu NISHIMURA, Jun-ichi KATO*, Masao YAMADA**
Hideho SUZUKI and Yukinori HIROTA†

Two temperature-sensitive (ts) mutants defective in chromosome partition have been described as *parA* and *parB* in *Escherichia coli*. They are characterized by growth into filamentous cells with centrally congregated nucleoids at restrictive temperatures. We found another kind of *par* mutants, JE11215 and JE10572, in the ts mutant stock of *E. coli* K12 (established with intention of the late Professor Y. Hirota to set up an exhaustive mutant collection for essential genes). The ts growth of these two mutants was corrected by pLC4-14 which is now known to cover the *metC* region at 65 min. The ts mutation in JE11215 was mapped at 65.3 min through the results of mating and P1-transduction experiments, being cotransducible with *metC* at 59% (29/49) frequency and with *glc* at 20% (5/25) frequency.

The ts mutation was transferred by cotransduction with *metC*$^+$ into a *metC* derivative of C600. The resultant ts transductants failed to grow on L-agar plates at 42°C and manifested a typical Par phenotype as observed

† See footnote to p. 39.

* Department of Bacteriology, National Institute of Health, Tokyo.
** Present address: National Children's Medical Research Center, Tokyo.

Table 1. Complementation of temperature-sensitive *par* mutations by plasmids carrying *dnaG* alleles.

Strains	Complementation by		
	pGL444::Tn5#4 (*dnaG*$^+$)	pGL444::Tn5#18 (*dnaG*$^-$)	pBS105::Tn5#128 (*dnaG*$^-$)
JE11215 (*parC*)	−	−	−
MFT100 (*parB*)	+	−	−
PC3 (*dnaG*)	+	−	−
MFT110 (*parA*)	−	−	−

Plasmids were a generous gift from Dr. J. R. Lupski and carry the *dnaG* segment with Tn5 insertion outside (*dnaG*$^+$) or within (*dnaG*$^-$) the *dnaG* gene (Lupski, J. R., *et al.*, 1982, *Mol. Gen. Genet.* **185**: 120).

with DAPI (4′,6-diamidino-2-phenylindole)-stained cells. This *par* mutation is neither allelic to *parA* nor to *parB* as described below and will be referred to as *parC*.

The *parA* mutation in MFT110 was previously reported to lie at 95 min. However, this turned out to be incorrect as described in the accompanying report (Kato, J. *et al.*, in this *Ann. Rep.* p. 41) and the map position of *parA* is unknown at present. The Par phenotype of the *parA* mutant MFT110 was not corrected by plasmids carrying the 65 min chromosome region and complementing *par* in JE11215. The *par* mutation found in JE11215 is clearly distinct from *parA*.

The *parB* mutation has been mapped between *thyA* (61 min) and *argG* (69 min). Although this mutation may be suspected to lie around the 65 min map position, the ts defect of *parB* mutant MFT100 was not corrected by pLC4-14 which complemented the *par* mutation in JE11215. Since *dnaG* has been mapped at 67 min and some *dna*^{ts} mutants can reveal rather Par-like morphology of nucleoids at restrictive temperatures, complementation of *parB* by the *dnaG* gene was investigated by transforming four relevant mutants, MFT100(*parB*), PC3(*dnaG*), MFT110(*parA*) and JE11215 with plasmids carrying *dnaG*$^+$ or *dnaG*$^-$:: Tn5. As shown in Table 1, the *dnaG* gene carried by the plasmid complememted *parB* as well as *dnaG*, but neither *parA* nor the *par* mutation in JE11215. These results indicate that the *par* found in JE11215 differs from *parB* and that *parB* is likely to be an allele of *dnaG*. More recently, it has been suggested by other authors that *parB* lies in the *dnaG* gene and affects primase (Norris, U. *et al.*, 1986, *J. Bacteriol.* **168**: 494).

The *parC* transductant, EJ812, with a genetic background of C600 grew into filaments with centrally located nucleoids at 42°C, releasing numbers of nucleoidless cells which were approximately of normal cell size. The fraction of nucleoidless cells accounted for more than 20% after 2 hr incubation at 42°C. The morphological phenotype was essentially unchanged by the introduction of *recA* into EJ812. Control of cell division by the process of chromosome partition seems to be independent of the *recA*-mediated division control. The production of nucleoidless cells by the *parC* mutant may suggest rather loose coupling between cell division and chromosome partition. The loose coupling might be specific to the *parC* mutation or alternatively inherent in *E. coli*.

Organization of the *parC-sufI* Gene Region in *Escherichia coli*

Jun-ichi KATO*, Yukinobu NISHIMURA, Masao YAMADA**
Hideho SUZUKI and Yukinori HIROTA†

The plasmid pLC4-14 which complements *parC* (for chromosome *par*tition) has been shown to correct by some degree the thermosensitive defect due to *ftsI* (mapped at 2 min, *pbpB* for *p*enicillin-*b*inding *p*rotein 3). The putative gene that can suppress the *ftsI* mutation is termed *sufI* (or *sui*).

The restriction map of pLC4-14 was determined as drawn in Fig. 1 and the *parC-sufI* region was analyzed by subcloning the chromosome region in it. Plasmids carrying the *Hpa*I fragment complemented *parC*, correcting both the thermosensitivity in growth and the impairment of nucleoid segregation in the hosts. Complementing ability was abolished by the insertion of a translation terminator into the *Pvu*II-2 site (Pv2 in Fig. 1). Thus, the *parC* gene lies on the *Hpa*I fragment. The allelism of $parC^+$ on pLC4-14 to *parC* was confirmed by cloning *parC1215* in JE11215. The plasmids that included the *Hpa*I fragment directed *in vitro* synthesis of the 75 kDa (75K) protein. When the translation terminator was inserted into the *Pvu*II-2 site, the 75K protein was not produced and a 69K protein was synthesized instead. Agreement between the complementing ability for *parC* and the synthesis of 75K protein supports the notion that the 75K

† See footnote to p. 39.

* Department of Bacteriology, National Institute of Health, Tokyo.
** Present address: National Children's Medical Research Center, Tokyo.

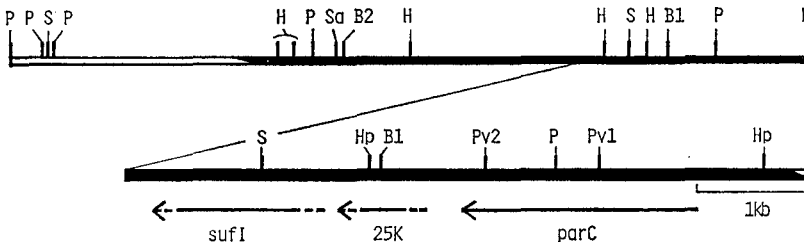


Fig. 1. The restriction map of pLC4-14.
Arrows indicate the location and the transcriptional direction of the genes for *parC*, 25K protein, and *sufI*. B1 and B2, *Bam*HI; H, *Hin*dIII; Hp, *Hpa*I; P, *Pst*I; Pv1 and Pv2, *Pvu*II; S, *Sma*I; Sa, *Sal*I.

protein is a *parC* product. A short deletion was introduced at the *Bam*HI site (B1 in Fig. 1) by BAL-31 digestion and subsequent insertion of a translation terminator. The *in vitro* product directed by the plasmid carrying this deletion showed an intermediate size between 75K and 69K. The 69K protein is most likely to be a curtailed *parC* and thus transcription proceeds from right to left in Fig. 1.

Plasmids carrying the *Bam*HI fragment suppressed *fts*I. The suppression occurred only under induction by IPTG (isopropyl β-D-thiogalactoside) when the B1 end of the *Bam*HI fragment was joined next to the *lac* promoter-operator region with a mini-F replicon. Accordingly, the transcriptional direction of the *sufI* gene is from right to left in Fig. 1 and the suppression of *ftsI* by the *sufI* gene results from an increase in the amount of *sufI* gene product. The *sufI* gene product was identified as a 55K protein synthesized *in vitro* under the direction of a plasmid carrying the *Bam*HI fragment. The production of the 55K protein was abolished, with the simultaneous loss of the ability to suppress *ftsI*, by inserting a translation terminator into the *Sma*I site within the *Bam*HI fragment. The 55K protein radiolabeled in maxicells was chased into a fast migrating component in SDS-electrophoretic gels, suggesting that the occurrence of posttranslational processing. The *sufI* gene appears to be dispensable for cell viability, since the *sufI* gene was inactivated without loss of viability through recombinational substitution with the *sufI* gene region interrupted at the *Sma*I site by the insertion of a *cat* gene.

However, the *Bam*HI fragment containing the *sufI* gene could not be deleted even in the presence of the same fragment supplied with a plasmid.

The possibility for essential genes covering the *Bam*HI sites was explored by insertional interruption at these *Bam*HI sites. A *polA*^{ts} strain N1126 was transformed with a derivative of pLC4-14 in which two different drug resistance genes were inserted into each of the *Bam*HI sites (B1 and B2 shown in Fig. 1). After the recombination process and plasmid curing at the *polA* restrictive temperature, drug resistant recombinants with insertion at the B2 site were obtained but those with insertion at the B1 site were not. Recombinants with insertion of a drug resistance gene at the B1 site could be isolated in the presence of a mini-F derivative carrying the *Sma*I fragment (Fig. 1). Viability of the isolated recombinants was lost through curing of the mini-F derivative. These results indicate the existence of an essential gene covering the B1 site. The product of this gene was identified as a 25K protein, the production of which was abolished by insertional interruption at the B1 site.

When the largest *Pst*I fragment of pLC4-14 was placed under the *lac* promoter, production of both *sufI* and 25K proteins was enhanced by the *lac* promoter ligated to the *Pst*I site within the *parC* gene. The *sufI* gene and the gene for 25K protein are transcribed in the same direction and thus they are possibly organized into an operon together with the *parC* gene at the proximal side. However, the *sufI* gene and the gene for 25K protein were significantly expressed by the *Pst*I fragment connected in reverse orientation to the *lac* promoter. There may be internal promoters for these genes.

The localization of the gene products was examined with maxicells by fractionating the sonicated lysates by ultracentrifugation in the presence or absence of Mg^{2+}. The *parC* gene product was found in the particulate fraction in the presence of 10 mM Mg^{2+} but was distributed in the soluble fraction in the presence of 10 mM EDTA. The 25K protein was found almost exclusively in the particulate fraction irrespective of Mg^{2+} availability. The majority of *sufI* gene product was present in the soluble fraction. Although it might be suspected that the *sufI* gene product is subject to processing and thereby released into the periplasm, its localization in the periplasm is not obvious at present. For details, see *J. Bacteriol.*, in press.

A Mutant Defective in the Processing of Penicillin-Binding Protein 3 of *Escherichia coli*

Hiroshi HARA, Yukinobu NISHIMURA, Seiichi YASUDA
Hideho SUZUKI and Yukinori HIROTA†

Penicillin-binding protein 3 (PBP-3) of *Escherichia coli* is a cytoplasmic membrane protein that plays an essential role in cell division. It functions in the formation of a septum of the murein sacculus, and is one of the lethal targets of β-lactam antibiotics. PBP-3 is a 588-residue polypeptide as deduced from the nucleotide sequence of its structural gene, *ftsI*, and is synthesized in a precursor from to be processed into a mature form distinguished by a higher electrophoretic mobility in SDS gels (Nakamura, M. *et al.*, 1983, *Molec. Gen. Genet.* **191**: 1).

We found two *E. coli* mutants, JE10092 and JE7304, which produced PBP-3 with a lower electrophoretic mobility than that of wild-type PBP-3. These mutants were investigated for possible defects in the processing of PBP-3. JE10092 was found in the temperature-sensitive (ts) mutant stock of *E. coli*. This strain was originally reported as a mutant with an altered RNA polymerase β′ subunit (Sugiura *et al.*, 1977, *Biochem. Biophys. Res. Comm.* **76**: 739) and then found to produce the altered PBP-3. Temperature-resistant (tr) transductants of this mutant showing tr RNA polymerase activity still produced PBP-3 with a lower mobility, indicating that the ts defect was associated with the defective RNA polymerase rather than due to an alteration in PBP-3. Introduction of plasmid pMS316 carrying the *ftsI* gene into this mutant resulted in the overproduction of PBP-3 of a normal mobility. When an *ftsI730* strain having the ts defect in PBP-3 was made tr by P1-transduction from this mutant, all the transductants produced mutant-type PBP-3. These results suggest that the lower mobility of PBP-3 in JE10092 is not due to a defect in the processing mechanism but to a mutation in the *ftsI* gene. The *ftsI* gene was cloned from JE10092 and analyzed for the gene product. A pulse-chase experiment revealed that the product was processed from the precursor form into the mature form and that both forms showed lower mobilities than those found in wild-type cells. The *ftsI92* mutation in JE10092 appears to result in the production of the altered PBP-3 which is larger in size but not defective in processing and functioning.

† See footnote to p. 39.

The other mutant, JE7304, producing PBP-3 with a lower mobility was found fortuitously in constructing multiple mutant strains for PBPs. This mutant showed ts growth at a low salt concentration. The tr transductants produced wild-type PBP-3, suggesting that the ts growth was possibly due to a mutation affecting the mobility of PBP-3. Preliminary experiments for mapping showed that this mutation (*ts-7304*) was not mapped in the *ftsI* gene. When pMS316 was introduced into JE7304, PBP-3 with a lower mobility was overproduced. Pulse-chase experiments with a *ts-7304* mutant harboring pMS316 showed that the processing of PBP-3 did not occur in this mutant. These results indicate that JE7304 has a defect in the processing mechanism of PBP-3.

The ts growth due to *ts-7304* was linked with a processing defect in PBP-3. Although this may suggest that the processing of PBP-3 is of vital importance, it is also possible that the ts growth is due to a failure in the processing of some essential protein(s) other than PBP-3. In either case, the present results suggest that the *ts-7304* gene is involved in an important processing system.

Processing in the C-Terminal Portion of Penicillin-Binding Protein 3 of *Escherichia coli*

Hiroshi HARA, Jun-ichi KATO*, Hideho SUZUKI and Yukinori HIROTA†

It is well known that precursor forms of proteins destined for extracytoplasmic locations are processed into mautre forms by proteolytic cleavage of N-terminal signal peptides during transfer across the cytoplasmic membrane. Penicillin-binding protein 3 (PBP-3) of *Escherichia coli* is a cytoplasmic membrane protein essential for cell division. Its catalytic site is likely to protrude into the extracytoplasmic side. It is synthesized in a precursor form and processed to yield a mature form (Nakamura, M. *et al.*, 1983, *Molec. Gen. Genet.* **191**: 1). Proteolytic cleavage may be involved in producing the mature PBP-3.

Two processing enzymes, signal peptidases (SPases) I and II, have been described in *E. coli*. SPase II cleaves signal peptides of prelipoproteins and SPase I processes many other extracytoplasmic proteins. The process-

† See footnote to p. 39.

* Department of Bacteriology, National Institute of Health, Tokyo.

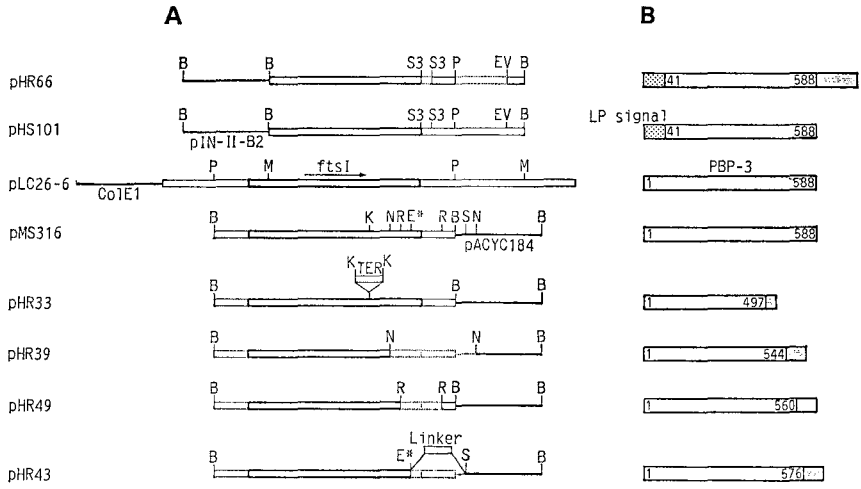


Fig. 1. Plasmids used in this study.

A, The restriction maps of the plasmids are shown with only the relevant restriction sites: B, *Bam*HI; E, *Eco*RI; EV, *Eco*RV; E*, *Eco*RI*; H, *Hin*dIII; M, *Mlu*I; N, *Nar*I; P, *Pvu*II; R, *Rsa*I; S, *Sal*I; S3, *Sau*3AI. TER, the synthetic translation terminator. Linker, the polylinker derived from pUC18. B, PBP-3 or its derivatives encoded in the plasmids are shown with the numbering of amino acids from the N-terminal methionine of PBP-3. Shadowed bars, the lipoprotein singal peptide lacking the processing site and followed by 7 residues due to a linker sequence in the pIN-II-B2 vector. Hatched bars, C-terminally extended peptides encoded in the DNA region fused to the *ftsI* gene. The figure is not drawn to scale.

ing of PBP-3 was not inhibited by globomycin, a cyclic peptide antibiotic which specifically inactivates SPase II, nor was affected in the *lsp* mutant defective in SPase II. Maltose-binding protein, OmpA protein and OmpF protein (processed by SPase I), and murein lipoprotein (processed by SPase II) were all normally processed in a *ts-7304* mutant defective in the processing of PBP-3. Furthermore, kinetic studies showed that the processing of PBP-3 was much lower in rate than the processing of other extracytoplasmic proteins. Thus the mechanism for maturation of PBP-3 seems to be distinct from processing mechanisms known so far.

The mode of processing for PBP-3 was investigated by manipulating the cloned *ftsI* gene. First, we examined whether the processing of PBP-3 occurs because of the removal of an N-terminal signal peptide by analyzing the product of pHS101 (Fig. 1). In this product, the N-terminal 40 residues

of PBP-3 were replaced by the murein lipoprotein signal peptide which lacked the processing site and was followed by extra 7 residues derived from a linker sequence in the vector. The pulse-chase experiment showed that this hybrid protein was processed. The processing was not inhibited by globomycin but prevented in the *ts-7304* mutant. It is unlikely that more than 40 residues are removed from the N terminus of PBP-3, since PBP-3 has only two cysteines at the 28th and 30th residues in the deduced sequence and yet the [^{35}S]cysteine label remained uneliminated in the mature PBP-3.

Next, we examined the C-terminally truncated PBP-3. A synthetic translation terminator was inserted into the *Kpn*I site within the *ftsI* gene to construct pHR33 (Fig. 1). This should result in the removal of C-terminal 91 residues of PBP-3. The truncated product was not processed in maxicells, although it seemed to be unstable and disappeared during the chase in growing cells. The truncated products which lost various lengths of the C-terminal part (Fig. 1) were then examined. The product directed by pHR39 was unstable not only in growing cells but in maxicells. The product directed by pHR49 was unstable in growing cells and not processed in maxicells like that directed by pHR33. The truncated product directed by pHR43 did not appear to be processed during the 30 min chase of growing cells pulse-labeled with [^{35}S]methionine, but was identified as two bands when the cell envelope was labeled with radioactive penicillin. The faster moving band was not detected in the envelope of the *ts-7304* mutant. These results indicate that the product directed by pHR43 is processed very slowly. In cells harboring pHR49, a single faint band of the product was detected in a penicillin-binding assay and the band position was the same for both the *ts*-7304 mutant and the wild type. Thus, we conclude that the peptide(s) essential for processing is contained in the C-terminal region between the 560th and the 576th residues.

To confirm the occurrence of proteolytic cleavage in the C-terminal portion, we constructed pHR66 in which the *Sau*3AI fragment was deleted from pHS101 in order to remove the normal termination codon (Fig. 1). This should allow the production of a molecule containing five cysteine residues only within the extra peptide tail added to the normal C terminus of PBP-3. The product directed by pHR66 was slowly processed as shown in a [^{35}S]methionine-labeling experiment, but the [^{35}S]cysteine label was not found in the mature form. This indicates that the C-terminal portion is removed during the processing of PBP-3.

In Vitro System for the Processing of Penicillin-Binding Protein 3 of *Escherichia coli*

Hiroshi HARA, Hideho SUZUKI and Yukinori HIROTA†

The mature form of penicillin-binding protein 3 (PBP-3) of *Escherichia coli*, a cytoplasmic membrane protein essential for cell division, is produced by proteolytic cleavage of the C-terminal portion of its precursor form as described in the accompanying report (Hara, H. *et al.*, in this *Ann. Rep.* p. 49). To investigate this unique processing reaction in detail, we tried to reconstitute the reaction *in vitro*.

When a plasmid carrying the structural gene for PBP-3, *ftsI*, was introduced into a *ts*-7304 mutant defective in the processing of PBP-3, a large amount of the precursor form was accumulated in the membrane fraction. This membrane fraction served as a "substrate" of the *in vitro* reaction. The membrane fraction prepared from an *ftsI730* mutant, in which the processing activity is normal but penicillin-binding ability of PBP-3 is lost, was used as an "enzyme". Two membrane fractions, "substrate" and "enzyme", were mixed and incubated in the presence of 0.2% Triton X-100 and then analyzed for PBP-3 through the binding of radioactive benzylpenicillin and subsequent electrophoretic separation on SDS gels. The precursor form in the "substrate" fraction was convered into the mature form. Thus the processing reaction could be reproduced *in vitro*. The *in vitro* processing was enhanced by nonionic detergents, Triton X-100 and n-octyl β-D-glucoside, but to a somwehat smaller extent with the latter. The detergent might help the interaction between the "substrate" and "enzyme" membrane vesicles. The addition of ATP showed no effect. Even under the optimal conditions and after a long period of incubation, at most half the amount of the precursor form was processed.

Proteins in the "enzyme" membrane fraction were solubilized with 2% Triton X-100 in the presence of 1 M NaCl, fractionated by ion-exchange chromatography with DEAE-cellulose and then with CM-cellulose. The fractionated proteins were assayed for processing activity by mixing with the "substrate" membrane fraction. The activity was recovered in the fraction which adsorbed to the CM-cellulose. Not more than half the amount of the precursors was processed by the solublized enzyme as observed for

† See footnote to p. 39.

processing by the "enzyme" membrane fraction. The processing site of the precursor molecules might be enclosed inside the membrane vesicles in half of the vesicle population. The substrate precursor form was similarly solubilized from the "substrate" membrane fraction and concentrated by adsorption to DEAE-cellulose. The solubilized precursors were completely inert for processing. The precursor molecules of PBP-3 might be altered in conformation when released from the membrane structure or might be susceptible to processing when incorporated into putative division complexes.

The *in vitro* processing system will be useful for biochemical analysis of the processing reaction and for purifying the enzyme for C-terminal processing.

Purification and Characterization of the *dnaA* Protein of *Escherichia coli*

Seiichi YASUDA

The *dnaA* protein of *E. coli* plays a central role in the initiation of chromosome replication. The *dnaA* gene has been cloned on a runaway replication plasmid and the *dnaA* protein has been shown to be overproduced in *E. coli* strains harboring the plasmid (Yasuda and Takagi, 1983, *J. Bacteriol.* **154**: 1153). In order to elucidate the functions of the *dnaA* protein, it was partially purified and characterized.

Cells having the *dnaA*-overproducing plasmid were lysed with the standard lysozyme method. Cell debris was removed by ultracentrifugation and the supernatant was fractionated by ammonium sulfate at 0.25 g/m*l*. The precipitates were redissolved and dialysed against a buffer solution containing 25 mM HEPES (pH 7.6), 5 mM $MgCl_2$, 2 mM dithiothreitol and 20% glycerol. The dialysed fraction was applied to a column of CM Sephadex C50 and the column was eluted with a linear gradient of 0–0.5 M KCl in the buffer mentioned above. The *dnaA* protein was eluted at about 0.25 M KCl. SDS-polyacrylamide gel electrophoresis of the *dnaA*-containing fraction showed that the *dnaA* protein (52 kdal) was the only major protein and was more than 70% pure.

Using this fraction the heat-stability of the *dnaA* protein was studied. As shown in Fig. 1, incubation of the *dnaA* protein in 25 mM HEPES pH 7.6 at 30°C for 10 min resulted in almost complete inactivation. The

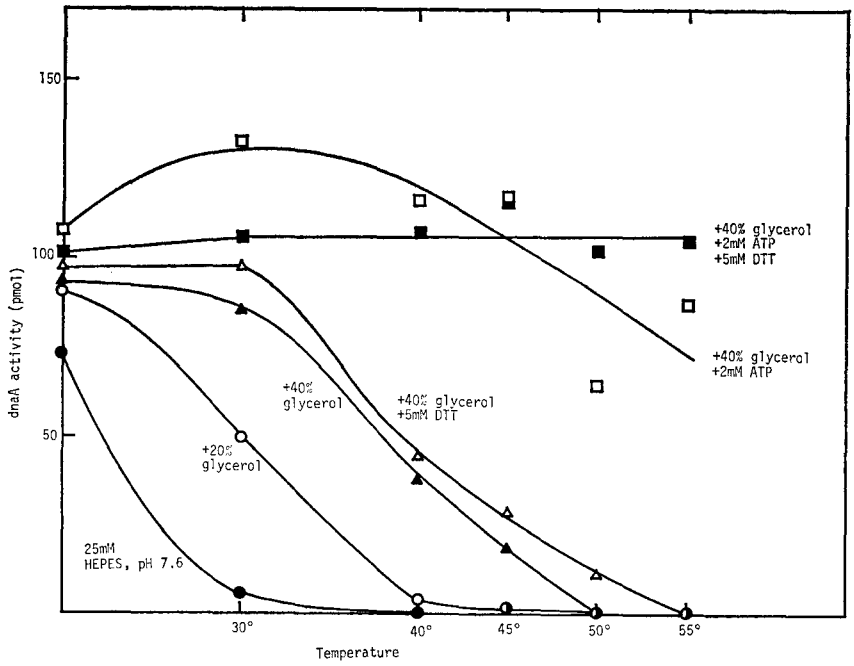


Fig. 1. Heat stability of the *dnaA* protein. Purified *dnaA* protein was diluted with 25 mM HEPES pH 7.6 containing various additions and heated for 10 min at temperatures indicated. The *dnaA* activity was assayed with the *oriC*-dependent replication *in vitro*.

addition of 20 or 40% glycerol had marked effects on the stability of the protein, but upon incubation at 40°C all of the activity was lost. A striking stabilization was observed when the *dnaA* protein was incubated with 2 mM ATP. The protein was still active even after heating at 55°C for 10 min in the presence of 2 mM ATP and 40% glycerol and there was no sign of inactivation by heating up to 55°C, if 2 mM ATP, 40% glycerol and 5 mM dithiotreitol were present in the *dnaA* protein solution. Stabilization by ATP means that the *dnaA* protein interacts with ATP and changes its conformation resulting in a heat-resistant form. There were no detectable DNA-dependent or independent ATPase activities in the *dnaA* fraction. Recently Sekimizu and Kornberg showed that *dnaA* protein binds ATP and ADP and that the ATP-bound form of the *dnaA* protein is active in rep-

lication reactions. Stabilization of the *dnaA* protein by ATP shown in the present study supports their results and may give a clue to understanding the *dnaA* protein function in the replication of *oriC* DNA.

Involvement of the *dnaK* Protein in Replication of *oriC* DNA *in vitro*

Yoshimasa SAKAKIBARA and Seiichi YASUDA

The *dnaK* gene of *Escherichia coli* encodes a heat shock protein of 70 kdal which is involved in DNA replication of chromosome and lambda phage. However, its precise function in chromosome replication has been unknown. Recently Sakakibara isolated a *dnaK* mutant (*dnaK111*) which is defective in the initiation of chromosome replication. In the present study we examined the participation of the *dnaK* protein in *oriC*-dependent replication *in vitro*. A crude enzyme fraction (fraction II) prepared from *dnaK111* mutant cells showed almost the same level of replication activity as that from wild type cells and there was only a little difference in heat-sensitivity between them. If fraction II's were prepared, however, from cells which had been heated at 42.5°C for 20–40 min just before harvest, the replication activity was much lower in the *dnaK111* mutant than in wild type cells. The defect in the *in vitro* replication of the *dnaK111* strain could be corrected by the addition of a small amount of fraction II from a strain which overproduces the *dnaK* protein. These results indicate that the *dnaK* protein is essential for the *oriC*-dependent *in vitro* replication reaction. A. Korhberg's reconstitution system, which supports replication of *oriC* DNA with only purified proteins, does not include the *dnaK* protein as a constituent, and hence, re-examination of the reconstitution system seems to be necessary.

Inhibitor of DNA replication in *Escherichia coli*, a Possible Regulatory Protein

Seiichi YASUDA

An *in vitro* replication system dependent on *E. coli* replication origin, *oriC*, was developed and analysed extensively by A. Kornberg's group. The reaction was first carried out with a crude protein fraction prepared by ammonium sulfate precipitation of cleared lysate of *E. coli* cells (fraction

II). Protein factors necessary for the reaction were fractionated and purified from the crude fraction and now it is possible to perform the reaction using only purified proteins (reconstitution system).

A striking feature of these reactions, both the reaction with the crude fraction and the reconstitution system, is their potent replication activities which are much higher than those of other *in vitro* replication reactions including bacteriophage DNA-dependent one. This is in marked contrast with the replication reaction *in vivo*; the chromosome is replicated only once in a generation (*ca* 20 min at most), whereas bacteriophage DNAs are replicated in an almost uncontrolled fashion.

From these results it seems to be reasonable to postulate that the *oriC*-dependent *in vitro* replication system lacks regulatory factors which are present in *E. coli* cells and which regulates replication in a negative way. To test this hypothesis experiments were carred out to detect in *E. coli* cell lysates a factor which specifically inhibits the *oriC*-dependent *in vitro* replication reaction.

Protein fractions were prepared from cleared lysates of *E. coli* cells by precipitation with various concentrations of ammonium sulftae. No significant inhibitory activity was found in fractions prepared by lysing cells in the presence of a low salt concentration. However, when cells were lysed in the presence of KCl at concentrations higher than 0.5 M, a considerable inhibitory activity on the *oriC*-dependent replication reaction was found in a fraction prepared by precipitation with ammonium sulfate added at 0.28 g per m*l* of cleared lysate. This activity was present in a strain defective in DNases and no exo- or endonuclease activity was found in this fraction, suggesting that the inhibition is not due to DNA degradation. Furthermore, the inhibitor was not active on ϕX174 single stranded DNA-dependent replication. This implies that the inhibitor is not a general inhibitor of DNA replication.

In order to characterize the inhibitor further, partial purification was carried out. The inhibitor was adsorbed to phosphocellulose and was eluted at 0.5 M KCl. This fraction, although not entirely pure, was used in the following experiments. The inhibitor was rather temperature-sensitive and lost 80% of its activity by heating at 65°C for 10 min. This temperature-sensitivity and the property to adsorb to phosphocellulose suggest that the inhibitor is proteinous in nature. The inhibitor acts at an early stage of *oriC* DNA replication, because addition of the inhibitor

after brief preincubation of the reaction mixture had no effect on the incorporation of nucleotides. The inhibitor was also found to be active on ColE1 DNA replication *in vitro*. It is not clear whether these inhibitory activities are due to a single protein or different ones. Further purification and assignment of its gene are in progress.

Mapping of a Whole Set of Cell Division Genes in *Escherichia coli* K12

Akiko NISHIMURA and Reiko IROBE

E. coli is supposed to have more than one hundred *fts* genes, which are involved in cell division. Up to now only twenty *fts* mutants among different genes have been identified. Two kinds of *E. coli* culture banks of this stock center have made it possible to identify a whole set of *fts* genes in a short time. One bank is Hirota's collection of 5000 temperature sensitive (TS) mutant strains, and the other is Clark & Carbon's collection (Neidhartdt *et al.* (1983), *Microbiol. Rev.*, **47**, 231–284) of 2000 strains which harbor ColE1 plasmids carrying small random segments of the *E. coli* chromosome (pLC-plasmids). Hirota's TS collection contains 410 unknown *fts* mutant strains, which grow normally at 30°C but make filament cells at 42°C because of a TS defect in the process of cell division.

Each of the 2000 streptomycin-sensitive F^+ strains containing pLC-plasmids was transferred via a metal replicating block with 48 needles from stock cultures maintained in Micro-Test III plates to L-agar plates. These master plates, after overnight incubation at 37°C, were replica-plated on lawns of above 410 each of streptomycin-resistant F^- TS (*fts*) strains on L-agar plates supplemented with 100 ug/ml of streptomycin. These plates were incubated for 2 days at 42°C to allow selective growth of recipients which had acquired a hybrid plasmid correcting the TS defect.

As a result, the TS defects of 350 *fts* mutants were corrected by 496 kinds of pLC-plasmids. Within those, 147 *fts* strains were complemented for the TS defect by pLC-plasmids whose molecular species of the inserted chromosome segments were analyzed already. Part of the results are shown in Fig. 1.

Interestingly each of the 27 *fts* mutants was complemented for the TS defect by 2 different species of pLC-plasmids, and 5 (4, 1) *fts* strains were complemented by 3 (4, 5) different species of pLC-plasmids respectively.

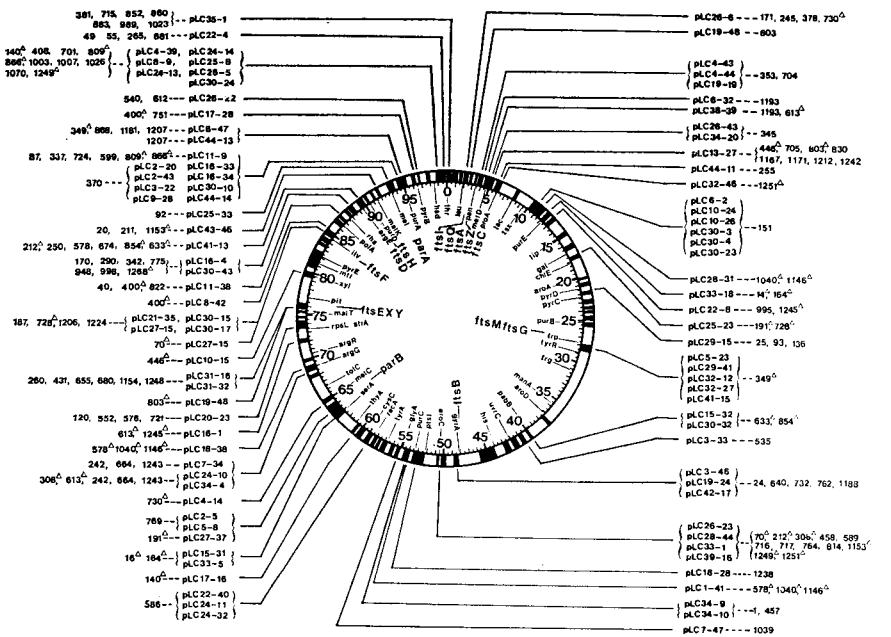


Fig. 1. Fts mutations in E. *coli* K12.

The outmost numbers show the strain number of *fts* mutants which was complemented for TS defect by the pLC-plasmids connected with a dotted lines. The plasmid information is from Neidhardt *et al.* (*Microbiol Rev.*, **47**: 231–284, 1983) and the gene information (in the inner part of circle) is from Bachmann (*Microbiol Rev.*, **48**: 180–230, 1983), but only *frs* mutants complemented by pLC-plasmids are shown. The dark blocks indicate chromosome segments inferred to be carried on the designated plasmids. The *fts* mutants which was complemented for TS defect by plural kinds of pLC-plasmids differed from each other in their chromosome moiety are marked by △ at the shoulder of *fts* strain numbers.

The *fts* mutants which were complemented for the TS defect by plural kinds of pLC-plasmids, differed from each other in chromosome moiety, are marked by △ at the shoulder of the *fts* strain numbers in Fig. 1. Transduction analysis by Pl-phage showed that TS mutation of those *fts* mutants was mapped at only one of those different loci. One pLC-plasmid contains the *fts* gene itself and the others are supposed to contain suppressor genes which could correct the TS mutation of *fts* only when the gene is

present in multiple copies in a cell (Takeda *et al.* (1981), *Plasmid*, **6**, 86–98). This kind of putative suppressor gene for *fts* mutations was found in 23% of the total analyzed.

The remaining 110 *fts* strains were complemented for the TS defect by one species of pLC-plasmid.

158 *fts* mutants were complemented for the TS defect by 345 pLC-plasmids, whose mapping position of chromosomal moiety are unknown. Continued research is necessary to determine the physical mapping position of those unknown pLC-plasmids by hybridization techniques using the gene library of Kohara's collection (Kohara *et al.* (1987), Cell, **50**, 495–508).

This research was supported by a Grant-in-Aid for Scientific Research on Priority Areas.

III. IMMUNOGENETICS

Sex-specific Recombinational Hot Spot in the Mouse Major Histocompatibility Complex

Toshihiko SHIROISHI, Naoto HANZAWA, Tomoko SAGAI and Kazuo MORIWAKI

Some Asian wild mice show increased recombination frequency within the MHC region in crosses with inbred strains. We previously reported one of such H-2 congenic strain, B10.MOL-SGR, whose H-2 was derived from Japanese wild mouse (Shiroishi *et al.* 1982, 1987). Any crosses between B10.MOL-SGR and inbred strains showed a 100 fold higher recombination frequency in the K-A_β interval of the MHC. Surprisingly, the increase in recombination was observed only in female meiosis.

In the present study, we mapped crossover points of fifteen independent recombinations which were generated from crosses between B10.MOL-SGR and inbred strains. Out of fifteen, seven were produced from crosses of B10/ B10.MOL-SGR and eight from crosses of B10.A/B10.MOL-SGR. We used polymorphic restriction sites between parental strains as genetic markers to locate the crossover points at the DNA level. Results indicated that all crossover points are mapped to a DNA segment of 30Kbp in length, which is located between the $A_{\beta 3}$ and $A_{\beta 2}$ genes. Nine of them were further confined to a short stretch of approximately 1Kbp DNA.

As previously reported, recombination involving B10.MOL-SGR shows a unique directional bias in the appearance of recombinant types depending on strain combinations of crosses. From crosses with the B10 strain, most recombinants retain K of B10 and A_β of B10.MOL-SGR, while reciprocal types scarcely appeared. On the other hand, from crosses with B10.A, recombinants preferentially retain K of B10.MOL-SGR and A_β of B10.A. Interestingly this directional bias reflected the fine locations of crossover points in the hot spot of 1Kbp DNA. All three recombinations which retain K of the B10 strain were confined to a 700bp DNA segment proximal to the MspI site in B10.MOL-SGR. Five recombinations which retain the K of B10.A strain were mapped to a 400bp DNA segment distal to the same MspI site. These results suggest that the recombinational hot spot of 1Kbp in length is split into two parts and each

combination of crosses favors a different part of the hot spot of 1Kbp DNA. Thus it became clear that B10.MOL-SGR has a female-specific recombinational hot spot in the $A_{\beta 3}$-$A_{\beta 2}$ interval of the MHC.

In order to analyze the molecular structure of this recombinational hot spot and to examine whether this segment has the consensus DNA sequence for the site-specific recombination, we attempted to clone the hot spot from B10.MOL-SGR and its counter regions from other parental strains, B10.A and B10, using a cosmid vector. So far we have succeeded in cloning these DNA segments of approximately 40 Kb in length from all three mouse strains. The DNA sequencing analysis around the recombinational hot spot is now proceeding.

Extensive Polymorphisms of the MHC Class II Genes in Japanese Wild Populations of Rat

Hideo GOTOH, Yoshi KAWAMOTO*, Toshihiko SHIROISHI and Kazuo MORIWAKI

The major histocompatibility complex of the rat (RT1), which is homologous to the HLA complex in humans and the H-2 complex in mice, encodes three sets of class II molecules referred to as RT1.B, RT1.D, and RT1.H. The polymorphism of the RT1 class II genes has been determined serologically in inbred strains of rat and in wild rat in Europe and the USA. Eight unique haplotypes have been described for RT1 class II genes in inbred strains of rat, and low polymorphism has been observed in wild populations of rat. We surveyed polymorphisms of RT1 class II genes in Japanese wild populations by restriction fragment length polymorphism analysis. The present preliminary survey discovered extensive polymorphisms in these populations. We confirmed that most inbred strains of rat originated from wild populations in Europe and the USA, and that many unknown RT1 class II haplotypes exist in Asian populations.

* Department of Animal Genetics, Faculty of Agriculture, Nagoya University,

Introduction of Human Steroid 21-hydroxylase Gene into Mice: An Attempt at Gene Therapy

Hideo GOTOH, Tomoko SAGAI, Toshihiko SHIROISHI and Kazuo MORIWAKI

We reported on an intra H-2 recombinant haplotype *aw*18 which truncates at about 80 kb in the H-2 S region. The functional steroid 21-hydroxylase (21-OHase) gene and the C4 gene are encoded in the deleted fragment. Steroid 21-hydroxylase acts in the cascade of adrenocortical steroidogenesis, and C4 protein plays a role in one of two pathways of the complement activation system. Homozygocity for the *aw*18 haplotype in mice is lethal in the early neonatal period. We assumed that the lethality of the *aw*18 homozygote is due to the inability to synthesize adrenocortical steroids in the mice.

So far, we have obtained 6 transgenic mice containing the human genomic 21-OHase gene with an H-2 genotype of either +/+ or +/*aw*18. If the introduced gene is expressed in the proper tissue, *aw*18 homozygotes would be rescued, and the mice should be a new and unique animal model for C4 deficiency.

Trans-subspecies Transmission of Mouse H-2 Polymorphism

Tomoko SAGAI, Toshihiko SHIROISHI and Kazuo MORIWAKI

Japanese wild mice, *M.m. molossinus*, have several genetic characteristics clearly distinguishable from European wild mice, *M.m. domesticus*, which are considered to be the ancestos of laboratory mice. Japanese wild mice were estimated to have a genetic distance of approximately one million years from European mice. Considering this unique feature of Japanese wild mice, we attempted to verify the hypothesis that H-2 polymorphism is older than at least the subspeciation of mice. Our early research focused on finding the H-2 antigens peculiar to Japanese wild mice and, in addition, the antigens common to European wild mice. Serological surveys of wild mouse populations using allo-antibodies indicated that three H-2K antigens, H-2K^{d}, H-2K^{f} and H-2K^{u} were predominant in Japanese wild populations. Their phenotypic frequencies were; d: 38%, f: 33% and u: 17%, respectively. Two of them, the d and f antigens, have been reported to be distributed widely in European and North American populations at

substantially high frequencies (Klein, J.). Therefore they seem to be common antigens preserved in both subspecies. On the other hand the H-2K^u antigen was found only in Asian populations including Japan, Taiwan and main land China. To investigate these common and population specific antigens in detail, we produced 10 monoclonal antibodies for the H-2K^f antigen and 4 for the H-2K^u antigen and made H-2 homozygous lines carrying wild-derived H-2 genes. Serological analysis using newly established monoclonal antibodies revealed several variations in antiganic determinants even in the same H-2K antigen which was identified by alloantibodies. It is, therefore, conceivable that each of the three H-2K antigens discussed above is not a single antigen but is part of a group including variants distinguishable by monoclonal antibodies. In addition to precise serological studies, we also carried out RFLP analysis for the wild mouse populations with the aid of a DNA probe to hybridize with the 3' non-coding region of the H-2K gene. This study indicated that each group of H-2K antigens such as d, f and u types, identified by serology, displayed representatively unique RFLP patterns. Of greater interest, the reactivity for only one of monoclonal antibody was consistent with the appearance of specific RFLP patterns in several cases. For example, the presence of determinant MS54, which is carried by the H-2K^f antigen group, always displayed the specific band irrespective of the presence of other determinants, while the H-2K gene identified as a member of the f group by alloantibodies did not show the specific RFLP pattern mentioned above if it did not have the MS54 determinant. Even out of the H-2K^f antigen group, if the MS54 determinant was present, the same specific RFLP pattern was obtained. These results may suggest that intra-genic recombination occurred in the middle of the H-2K genes to split the antigenic determinants into two parts, MS54 and f-specific determinants on the external domain of the H-2K molecule.

In conclusion the present studies demonstrated that common types of H-2K antigens beyond the subspecies barrier exist, although minor variations have accumulated in each of the subspecies. Thus it became clear that the ancestral f allele was present before subspeciation between Japanese and European wild mice.

IV. DEVELOPMENTAL AND SOMATIC CELL GENETICS

Preservation of Frozen *Drosophila* Embryos after Irradiation by Laser Microbeam

Yukiaki KURODA, Yuko TAKADA and Takahiro KASUYA*

Most stock cultures of *Drosophila* are being maintained in laboratories of universities and institutes at a temperature of 25°C or slightly lower by mating male and female flies. The day-to-day operation for maintaining these stocks entails enormous cost, labor, and time. In the present study, the possibility of using the laser microbeam to preserve early embryos of *D. melanogaster* at −196°C was examined.

The most difficult problem encountered in freezing *Drosophila* embryos has involved a method in which a protective agent is introduced into the eggs through the vitelline membrane which is very tolerant to chemical enzymes. In the present study, a UV laser microbeam was applied to inflict breaks or cuts on the micropile of the eggs in order to allow protective glycerol to enter.

Eggs were dechorionated by treatment with 3% sodium hypochloride solution. Eggs of the appropriate developmental stage were transferred to thick plastic slide plate, and then the plate was placed on the stage of a microscope, which had been incorporated into a laser microinjector system. A laser beam with a spot size 0.5 μm was focused through an incident-light microscope objective onto the micropile of each egg. Exact aiming was accomplished by pointing the target image on a TV monitor screen with a light pen.

The irradiated eggs were incubated at 25°C in Medium K-17 supplemented with fetal bovine serum and 15% glycerol. Following a 1–24 hour incubation, the eggs were frozen rapidly at −196°C in small glass vials in liquid nitrogen. After at least another 24 hours, the eggs were thawed and incubated in a salt solution at 25°C. A few of the eggs hatched, and the larvae grew to adult flies. Glycerol incorporation was confirmed by staining the eggs with neutral red. The survival rate of frozen, irradiated eggs

* Microbeam Physics Laboratory, The Institute of Physical and Chemical Research, Wako, Saitama.

increasing upon incubation with glycerol for at least 16 hours. The stage and site of eggs to be laser irradiated, the conditions of incubation, and the rate of freezing and thawing are now under investigation so as to yield a large number of viable embryos.

Studies on Sex Differentiation of Embryonic Cells of *Drosophila melanogaster*

Yukiaki KURODA, Bungo SAKAGUCHI* and Kugao OISHI**

In *Drosophila melanogaster*, a cluster of several cells which develops to germ cells in adult flies appears as the polar cells outside of the blastoderm at the posterior end of blastoderm embryos. Other morphological differences between male and female individuals can be distinguished only after pupation in normal development. No practical procedures have been found to detect the differences between somatic male and female cells during embryogenesis.

Sex-ratio organisms (*Spiroplasma SRO*) of *Drosophila* have male-specific lethal effects and kill only male embryos in early development. We established an experimental system in which undifferetiated cells dissociated from post-gastrula embryos differentiated into larval-type cells or adult-type structures in the absence or presence of ecdysterone in culture medium. In the present study we examined when SROs infect undifferentiated cells obtained from post-gastrula of *D. melanogaster*, how they recognize somatic male and female cells in culture, and what types of cells or tissues are affected by SROs.

A haemolymph of 0.2 m*l* containing SROs was collected by a glass capillary from abdomens of 300–600 adult flies 7 to 10 days after eclosion. The haemolymph was diluted with the same volume of salt solution, filtered through a Millipore filter and added to culture flasks in which embryonic cells had previously been cultured for 24 hours. After cultivation for one or two days at 25°C, SRO-infected cells were observed under an inverted phase microscope.

As a result, necrosis was found in some cells, suggesting that SROs hove lethal effects on infected cells. The necrotic cells appeared as brown clusters. The tissue specificity of affected cells are under investigation.

* Department of Sericulture, Faculty of Agriculture, Kyushu University, Fukuoka.
** Department of Biology, Faculty of Science, Kobe Univeristy, Kobe.

Injury Effect in Hydra Head Regeneration

Hiroshi SHIMIZU and Tsutomu SUGIYAMA

Roles of "injury effect" in hydra regeneration was examined. Strain reg-16 is a mutant strain which has a reduced head regenerative capacity. Kobatake and Sugiyama (1988) have shown that head regeneration by this strain can be significantly improved by the reopening of wounded tissue located at the apical regenerating end of the decapitated polyps. This suggests that injury inflicted upon the regenerating tissue has a positive effect on head regeneration in strain reg-16.

The present study was carried out to examine whether or not a similar effect of injury can be also demonstrated in head regeneration of the wild type strain of *Hydra magnipapillata* (strain 105). Head was removed by surgical amputation at midgastric region of an animal bearing the first bud protrusion. The decapitated polyps were then devided into three groups. In the first group (control), wound opening produced by amputation was allowed to close and heal normally. In the second group, wound opening was prevented from closing and healing for 24 hr by placing a nylon fishing line through the gastric cavity of the decapitated polyp. In the third group, wound opening was covered immediately after amputation. This was achived by grafting two decapitated polyps together in a mirror image symmetry in such a way that the wound opening of the two polyps covers each other.

It was found that the rate of head regeneration was significantly lower in the third group (mirror image grafting) than in the other two groups. Approximately 40% of the animals in this group did not regenerate a hypostome or tentacles, whereas nearly 100% of animals regenerated normally in the other groups. Furthermore, decapitated polyps whose wound closing and healing was delayed (second group) regenerated a significantly higher number of tentacles than in the control polyps.

These observations suggest that surgical removal of head alone does not necessarily lead to head regeneration, and that the state of injured tissue in the period immediately following head removal has a significant effect on determining regeneration. The underlying mechanism responsible for this effect is not well understood at present, but probably identical to the "injury effect" observed in hydra tissue transplantation phenomena by MacWilliams (*Devel. Biol.*, **96**, 239–257 (1983)).

Role of Interstitial Cell Migration in Generating Position-Dependent Patterns of Nerve Cell Differentiation in *Hydra*

Toshitaka FUJISAWA

Nerve cell differentiation in *Hydra* occurs predominantly in the head and foot regions. The role of interstitial cell migration in the formation of this position-dependent pattern was investigated in the regenerating head.

When distal tissue was removed from the body of wild type strain of *Hydra magnipapillata*, 105, nerve cell differentiation occurred at a rapid rate during the first 48 hr of regeneration, slowing after this point. The rapid nerve cell differentiation was due primarily to the migration of interstitial cells, some of which were nerve cell precursors. The migration, however, did not continue but decreased considerably after the first 48 hr of regeneration. In a mutant strain, reg-16, deficient in head regeneration, no migration of interstitial cells and hence no new nerve cell differentiation was observed in the regenerating tip. However, the interstitial cells of reg-16 were found to migrate if they found a proper environment. These observations suggest that the migration of nerve cell precursors plays an important role when the new nerve net is being rapidly established, e.g. head regenevation.

Genetic Studies on the Lifespan of Adult Silkworms Effect of Removal of Brain

Akio MURAKAMI and Jun SHIMADA*

The mean lifespan of adult silkworms was found to differ markedly with the strain under experimental conditions (25°C), and the lifespan of females was 1.5 times longer than that of males. The mean lifespan of females was about 10 days, but the lifespan of the *Daizo* strain females was 1.5–2 days, the shortest lifespan among silkworm strains. The hybrid females were generally longlived, and this characteristic was found to be dominant over short lifespan. The relationship between lifespan and brain function has been discussed for a long time. The brain-central nervous system plays an important role in basic or fundamental processes. Operations on the

* Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tsukui, Kanagawa-ken.

brain-central nerve system can be performed easily in silkworms. We examined the effect of brain removal on the mean lifespan of *Daizo*, the strain with the shortest lifespan, that of the *J106* strain showing a mean lifespan and the hybrid between the two strains.

The experiment was done on 100 silkworms each in the untreated control and brain removal groups for each strain and both sexes. The operation was conducted on pupae of both sexes just after pupal ecdysis up to 1 hour after pupation, in a simple clean bench. As a result, the operation was successful in about 75% of the silkworms which grew into adults. The removal of the brain shortened the lifespan in all strains and both sexes. A difference between the sexes was seen in *Daizo*, the short lifespan strain, but the effect of brain removal on the reduction of lifespan was small. The lifespan of females was reduced significantly by brain removal to a length of lifespan equal to that of untreated males. Males usually lived for 1–1.5 days, so the reduction was slight. However, in the F_1 hybrid obtained from a cross with *J106*, the effect of brain removal was marked in both sexes. The lifespan of the F_1 hybrid which was classified into the longest lifespan group, was reduced by about 2–3 days on the average. The males in both strains had a lifespan of about 2 days, and the removal of the brain had less effect on the lifespan of males than on that of females.

In conclusion, brain removal was found to reduce lifespan by about 2 days irrespective of the adult duration specific to the strain or sex. That is, the lifespan specific to the strain and the sex of adults depends on brain function in each strain and sex.

Genetic Studies of Parthenogenesis in the *Bombyx* Silkworm: Genetic Factors Responsible for the High Frequency of Spontaneous Parthenogenesis

Akio MURAKAMI

In the domesticated silkworm (*Bombyx mori*), naturally-occurring parthenogenesis, a form of asexual reproduction, is a popular phenomenon with a marked difference among strains or races ranging from a few percent to 70% or more. Particularly, an inbred line of the *Cambodge* strain, one of the tropical multivoltine races, showed a high potency (70% or more) for spontaneously-occurring parthenogenesis among two dozen silkworm strains investigated so far. A similar observation with an Indian com-

mercial multivoltine strain, *Nistari*, had been reported by Chowdhury (1953).

Most eggs laid by virgin *Cambodge* female moths developed spontaneously into, at least, the completion stage of organogenesis, while only 10% of the eggs from virgin females in various other strains, *Daizo*, *Aojuku*, *C108*, *J106 etc.*, attained the same embryonic stage as the *Cambodge*. However, the rest of the parthenogenetically developed eggs generally died before the completion stage of organogenesis regardless of strain or race. Many eggs laid by virgin moths showed no external indications parthenogenetic development (Murakami *et al.*, 1988). In *Bombyx mori*, the developing eggs are easily discernible from non-developing egg at the stage coloration with pigment granules in the serosa membrane cells. Developing eggs become dark gray, while non-developing ones remain light yellowish-white. Accordingly, it has been suggested that the multivoltine race may have a higher parthenogenetic potency than uni- or bivoltine races.

Eggs laid by F_1 female moths of a cross between the *Cambodge* females with a high frequency and *J106* males with a low frequency did not show a high incidence of spontaneous parthenogenesis. However, eggs laid by the F_1 moths of the reciprocal cross, *J106*♀ × *Cambodge* ♂, showed a frequency similar to that of Cambodge females. This clearly indicated that the genetic factor(s) responsible for a high potency for spontaneous parthenogenesis in the *Cambodge* strain is inherited autosomally and is not sex-linked. Vrigin female moths of the backcross F_1, (*Cambodge* ♀ × *J106* ♂) ♀ × *Cambodge* ♂, produced different egg batches with high and low frequencies for spontaneously-occurring parthenotes. The frequencies of parthenotes detected in the high line roughly corresponded to those of inbred *Cambodge* strain moths, while those in the low lines corresponded to those of the *J106* strain or about one fifth or one tenth of the *Cambodge* strain. A similar situation was also observed in egg batches laid by the reciprocal cross mentioned above.

It is of further interest to note that a ratio of low to high lines was nearly one to one, suggesting that a hereditary factor for the high parthenogenetic trait in the *Cambodge* strain may monogenic and may be located on a certain autosome. Virgin female moths of the BF_1 produced only one type of egg batch with a somewhat higher frequency in the event than that of the *J106* strain. Egg batches laid by F_2 moths of a cross between the *J106* females and *Cambodge* males were two types with high and low frequencies

of spontaneously-occurring parthenotes having a ratio of one to three. The present observations and others suggest that in the *Bombyx* silkworm, the tropical multivoltine race possesses a high potency for parthenogenesis compared with the uni- and/or bivoltine races.

Secretory Mode of Fibrous Cuticle (FC) in Silkworm

Tadasu MORI*, Jun SHIMADA** and Akio MURAKAMI

In insect integument, new cuticle is formed at every occurrence of ecdysis. In such a cuticularization in the fifth instar larvae of silkworm, the number of fibrous cuticle (FC) layers (about 15 just after the fifth ecdysis) increases to about 70 when the larvae grow for 7 days with an increase in thickness from 10 to 40 μm. In addition, an S-shaped profile can periodically be observed in such a thickening process. However, nothing has been reported so far on detailed electron microscopic observations of the FC secretion in insects, including silkworm, except for an assumption based on a study using electron microscope autoradiograph, that raw materials of FC are secreted (supplied) from epidermis (EP) in a desert locust.

In the present study, the FC formation process was observed very clearly under an electron microscope on a boundary section between normal and transparent integuments on the ventral side of the fifth segment of a threeday-old fifth instar mosaic larva obtained from a cross of *mo*/*mo*: *ok*/*ok* ♀ and +/+: *ok*/+ ♂. Compared with a normal individual, no differences were found in mosaic larvae in cell adhesion and structure of cuticle in the boundary section between normal and transparent EPs. In these two EPs at this stage of mosaic larvae, however, the following phenomena were observed: 1) Golgi bodies were found everywhere and were constructed by electron-dense spheres (EDSs) covered with a membrane; 2) these EDSs were transferred toward peripheral sites in cytoplasm, where the EP adjoins the cuticle layer, and are located densely in the site; 3) some of the these EDSs were released from the cells (into a space between EP and FC) by exocytosis; 4) at the released space, some or entire EDSs were metamorphosed into a fibrous mass; and 5) the metamorphosed fibrous mass moved into the FC which adjoined EP and was combined with FC.

* Kinjyo University, Nagoya.
** Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tsukui, Kanagawa-ken.

These phenomena observed in the EPs clearly differ from the secretory mode of ecdysial droplets whose secretion starts at the beginning of spinneret pigmentation. On the basis of the above results, it was found that the secretory mode of FC consisted of two processes: a secretory process (construction of EDSs as a raw material for cuticle-formation by Golgi bodies and release of EDSs outside the cells by means of exocytosis) and a formation process (metamorphosis of released EDSs into fibrous material, and incorporation of the material into FC and formation of fresh FC).

On the Sex of Artificially-Induced Parthenotes after Treatment of Newly Laid Unfertilized Ova with Hot-Water in *Bombyx mori*

Akio MURAKAMI

Meiosis is an important event in the course of gametogenesis and is composed of the two successive divisions. The majority of biological organisms are the pre-reductional type: the first division is a reductional (heterotypic) process from tetrad to dyad chromosomes and the second division is an equational (homeotypic) process from dyad chromosomes to a monad chromosome. The other is the post-reductional type: the first division is of an equational and the second is of a reductional. The *Bombyx* female is a heterogametic sex, designated in terms of the sex chromosome consitution ZW, and the male is ZZ. In the case of the pre-reductional division type, all artificially-induced parthenogenetic individuals produced by suppression of the second division would become exclusively ZZ males, since WW females are theoretically nonviable, whereas in the case of post-reductional type, parthenotes would result in only ZW females. Several attempts have been made in spite of serious difficulties to cytologically analyze the maturation divisions of spontaneous parthenogenetic eggs and have indicated that in naturally occurring parthenogenetic eggs, both maturation divisions take place (*e.g.*, Astaurov, 1967). Either non-seminated or inseminated ovum in the ovaries of adult moths corresponding to late prophase I oocytes or those of the preceding stage prior to the resumption of the 1st meiotic division. Anaphase I resumes while the pass through the ovaries. Newly deposited eggs on the egg-card are at anaphase I and immediately undergo telophase I about 10 min thereafter. The completion of the 1st division occurs *ca.* 20–30 min after oviposition. Successively,

the secondary oocyte goes on to interphase II and the 2nd meiotic division appears 90–100 min after oviposition. The completion of the 2nd meiotic division occurs about 10–20 min thereafter.

To clarify which meiotic division type the oogenesis of the *Bombyx* silkworm fits, 0–1 and 5 day-old ova (or secondary oocytes) laid from virgin F_1 female moths of a cross between *J106* and *Cambodge* males were collected and immersed for 15 min into hot-water kept at 46°C. After treatments the ova were then transfferred to water kept at a room temperature of 25°C for 15 min. The eggs were taken from the water and dried naturally in a temperature controlled room at 25°C and 60–70% relative humidity. Those artificially-induced parthenogenetic eggs in a diapause state were kept under natural conditions until the next generation. A very small number (less than 0.1%) of the diapausing parthenogenetic eggs were hatched regardless of the treated age of unfertilized eggs. Most hatched insects were grown until the last instar. All larvae observed showed female sex characteristics. Judging from morphological inspections of eggs and/or of larvae produced by the parthenogenetic females which had already been mated with normal diploid males, most (95% or more) of the parthenotes were regarded as diploid, and the rest as triploid. If the secondary meiotic division were blocked in the case of pre-reductional division types the majority of parthenotes would be appeared in the case of the post-reductional type, Thus, the present findings suggest that the oogenesis of *Bombyx mori* follows the post-reductional division process.

Embryonic Development of a Maternally Affected Embryonic-Lethal Mutant in *Drosophila melanogaster* (*II*)

Kiyoshi MINATO and Masa-Aki YAMADA

In the previous study on external morphology observed under an optical microscope, eggs laid by female flies homozygous for a maternally affected embryonic-lethal gene, fs(1)MAY-263 (Yamada, M. A., 1978) in *Drosophila melanogaster*, proved to develop into embryos with some teratogenic anomalies possibly caused by abnormal morphogenesis at an early stage of development. To investigate in further detail, histological preparations of lethal embryos at various stages of development were made to compare with those of normal embryos. Results showed from external morphology, that embryos developed normally until the end of the cellular blastoderm stage,

but thereafter, the formation of the inner layer of the germ band produced by the formation of the ventral furrow was not seen in mutant embryos. Instead, many transverse invaginations of the external layer, which were never seen in normal embryos, were formed here and there in abnormal embryos. The dorsal extension of the germ band did not occur and the posterior midgut rudiment appeared to invaginate at the posterior pole of embryos.

At a later stage, the abnormal embryos had the hypoderm constricted here and there possibly owing to the remnants excessive furrows in the external layer formed at the early stage. In those embryos, the visceral nerve system was formed but somewhat disorganized by the constrictions of the hypoderm. The muscle and fat body were scarcely seen in those embryos, and the midgut remained as a sac-like one without developing into a coiled gut.

It is interesting that the abnormal morphogenesis seen early in the development of the mutant embryos and the formation of excessive transverse invaginations of the external layer without the formation of the ventral furrow resulting in the formation of an inner layer of germ band are not an isolated cases but are seen in many lethal mutants, maternally or zygotically. The above syndrome commonly seen in these mutants may give us some important information about morphogenesis at early stages of embryonic development in *Drosophila*.

Isolation and Characterization of a Maternally Affected Embryonic-lethal Mutant Rescued by Transplantation of Normal Egg Cytoplasm in *Drosophila melanogaster*

Masa-Aki YAMADA and Saburo NAWA

In order to study the effects of the oocytoplasm on the gene expression of zygotonucleus in the early development in *D. melanogaster*, maternally affected embryonic-lethal mutants (29 complementation groups) were isolated through the treatment of X chromosomes with EMS. From these mutants, a mutant (*fs*(1)*MY*-18) rescued by the transplantation of normal egg cytoplasm was obtained. This mutant gene was located at 1–12.5 and located in 4F7-8–5A1-2 bands on the salivary gland chromosome. Homozygous females had normal viability (over 90% when compared to hetero-

zygous females) and the sex ratio of progeny produced by heterozygous females was 0.52. Homozygous females were anatomically normal. Although they were completely sterile, they had normal ovaries and produced morphologically normal eggs. However, these eggs were lethal when fertilized with sperm bearing a normal allele. But, the progeny (2% of treated eggs) were rescued with transplantation of the cytoplasm of normal eggs. These results suggest that this mutant gene had no effect on late development and the viability of the fly but was effective on the generation of oocytoplasm concerned in the early development of eggs during oogenesis.

Embryonic developmental features of this mutant were observed by time-lapse photographs taken through a phase-contrast microscope. Eggs laid by homozygous females developed normally to the syncitial blastoderm stage, but at the cellular blastoderm stage the formation of pole cells was reduced compared to that in normal embryos. Thereafter the extention of the germ band was weak and therefore the anterial elongation of the dorsal plate stopped at about 50% of egg length, although in normal embryos the tip of the dorsal plate reached about 75% of egg length and hence, little formation of the cepharic furrow was observed. The formation of the crypeolabrum and the mid gut was later than in normal embryos. Although the embryos moved actively within the vitellin membrane, they never hatched. They also had a normal cuticular pattern. These observations suggest that the maternal expression of this gene is required for the morphogenetic movements of embryonic cells in the early development.

A Novel Mobile DNA Element Consisting of Tandem Repeats at the White Locus of *Drosophila simulans*

Yoshihiro H. INOUE and Masa-Toshi YAMAMOTO

According to the detailed characterization of individual mobile elements isolated from *D. melanogaster*, they are classified into four families, *copia*, *P*, *FB* and *F*. It has also been demonstrated that some mobile elements such as the *copia*-group, are commonly found in various species belonging to the *D. melanogaster* subgroup. However, those which are species specific or restricted to only a few species have also been reported.

white-milky (w^{mky}) was the first mutant of *Drosophila simulans* demonstrated to be moderately unstable, and to carry a 16 kb DNA sequence inserted at the *white* locus. The spontaneous occurrence of w^{mky} and its revertability

suggest that the long insertional DNA belongs to a class of mobile elements. We cloned the entire insertional sequence and analyzed the molecular structure. The insertional DNA consisted of three tandem arrays of approximately 6.2 kb in size, although the most proximal repeating unit to the centromere had 2.5 kb deleted at the end. There were no detectable sequence homologies between both termini. It is not yet understood whether the basic structure of the mobile element is 6.2 kb or 16 kb, since variously fragmented internal sequences of the element exist in the genome. Since no homology was detected with some mobile elements isolated from *Drosophila*, the insertional DNA sequences were considered to be a novel dispersed repetitive DNA element, and named "ninja". This element exhibited intra-specific variation in genomic copy numbers.

Genetics of an Unstable *white* Mutant in *Drosophila simulans*: Reversion, Suppression and Somatic Instability

Yoshihiro H. INOUE, Toshifumi TAIRA and Masa-Toshi YAMAMOTO

A spontaneous *white* mutation, *white-milky* (w^{mky}) of *D. simulans* is moderately unstable and is associated with a 16 kb long DNA insertion into the *white* gene. w^{mky}, which is an unstable mutation found in *D. simulans*, has been genetically analyzed. Among nine spontaneous, partial reversions toward wild type, five were *white* locus mutations. They are phenotypically different from each other and three show eye color sexual dimorphism indicating a failure of the dosage compensation mechanism. Two *w* locus mutations whose eye color appeared identical between males and females were also isolated. Of the other back-mutants, three were associated with a recessive suppressor of w^{mky} and one was a semidominant suppressor. These suppressor loci are located on the third chromosome at map positions of about 90 and 120 respectively. The suppressor mutations demonstrate specific effects on *w* locus mutations derived from w^{mky} which lack gene dosage compensation. Somatic instability was detected at a frequency of 5.6×10^{-4} in w^{mky} flies heterozygous for the recessive suppressor and the frequency was increased 10-fold when the suppressor mutation was placed in a different genetic background.

The *ninja* Mobile Element is Abundant Specifically in a Strain of *Drosophila simulans*

Masa-Toshi YAMAMOTO and Yoshihiro H. INOUE

Debates on the evolutionary history of mobile DNA elements, continue and experimental evidence available to date upon which the major hypotheses stand do not seem supportive enough to reach a firm conclusion. For elucidating the origin and subsequent dynamics of mobile elements, a strain with specific dispersed repetitive DNA sequences is of crucial importance.

We previously identified a 16 kb mobile element, *ninja*, in a moderately revertable mutant, *white-milky* (w^{mky}) of *Drosophila simulans*. The entire insertional DNA, *ninja*, was isolated and the molecular analysis indicates that *ninja* is a mobile element whose structure is a novel type consisting of tandemly triplicated DNA sequences with no terminal repeats larger than 100 bp. *Ninja* did not show sequence homology for most of the well characterized mobile elements isolated from both *D. melanogaster* and *D. simulans*. We examined the DNA sequences homologous to *ninja* in nineteen *D. simulans* strains (Kl, w^S, $w^{2.4C}$, w^R, w^l, w^{mky}, w^{mky}-*2*, w^{NIG}, w^L, Leiden, Riverside, *y wf*, Rakujuen, O–9, SM1, K3, All Saint and $\widehat{XX}$, *y wf*), six strains of *D. melanogaster* (cn^1 bw^1, cn^{38j} bw^{38j}, Canton-S, M56i, St Hellens and Uman) and six species of the *melanogaster* subgroup (*D. mauritiana*, *D. sechellia*, *D. teissieri*, *D. yakuba*, *D. erecta* and *D. orena*) by either Southern blot hybridization or *in situ* hybridization to the salivary gland chromosomes. The results consistently showed that *ninja* DNA is abundant specifically in the w^{mky} strain, and the copy number in other strains is miniscule. Of significance is strain specificity in such a proportion that only one out of nineteen *D. simulans* strains posseses *ninja* in copy numbers. It is therefore very likely that the dispersed *ninja* elements in w^{mky} have evolved very recently in evolutionary history.

Grandsonless Strain of *Drosophila simulans* Carries Maternally Inheritable Sexratio Factor which Specifically Dysfunctions Y-bearing Sperms

Masa-Toshi YAMAMOTO and Takao K. WATANABE

Prezygotic "sex-ratio" traits so far isolated from wild populations of

various species of *Drosophila* in most cases deviated toward the direction favoring females, and the genetic factors responsible for the deviation were linked to either the X chromosome in the *D. obscura* species group or the 3rd chromosome in *D. simulans*. Postzygotic modifications of sex ratio come a wide variety of develpmental problems associated with sex specific gene regulations, such as a series of genes involving sex determination and dosage compensation mechanisms. In addition to the above examples of chromosomal sex-ratio genes, cytoplasmic factors, which distort the sex ratio postzygotically, have also been identified as mycoplasmas and labeled as sex-ratio organisms (SRO). However, cytoplasmic factors which strongly modify sex ratio *prezygotically* have not been previously reported.

In *Drosophila simulans*, we found a new type of sex-ratio distorter strain, and studied its genetic and cytological characteristics. Crosses between wild type males and females who are carriers of the distortion factor produced progeny at a sex ratio 1: 1. The daughters, when crossed with wild type males, produced equal numbers of males and females, but the sons produced predominantly female offspring. Effects of the distortion factor are phenotypically expressed in the F2 generation, and thus the number of grandsons is reduced significantly to about 10% of the total number of progeny. We call the strain *Grandsonless* (*GS*). The factor causing the phenotype, Grandsonless factor (*Gsf*), is maternally transmitted and not a chromosomal genic property. Being different from SRO, the factor seems resistant to the antibiotic tetracycline.

Spermiogenesis was examined in males produced by a mother who was a carrier of the factor *Gsf*. In the strain exhibiting the extreme distortion, the number of sperm heads were reduced to about half of the normal number of sperm in a sperm bundle. Since the first and the second meiotic divisions are confirmed to be normal cytologically, the distortion is attributed to prezygotic breakdowns of Y-bearing sperms at a stage of sperm elongation in spermiogenesis. Normal "egg to adult" viability supports this cytological observation.

We are currently in the process of isolating the cytoplasmic factor *Gsf* from the strain.

Studies on Gene Expression in Mouse Cerebellum

S. AOTA and T. IKEMURA

Gene expression in mouse cerebellum was studied using a mutant mouse (*nervous*) that is known to be deficient in normal cerebellum development. We constructed a cDNA library from normal mouse cerebellum and isolated clones that are specifically expressed in cerebellum (e.g., not expressed in cerebral cortex and in liver) but not in the *nervous* cerebellum. These clones are useful developmental markers for the cerebellum. We determined nucleotide sequences of several cDNA clones thus isolated and found that one clone has a significant homology for an amino acid sequence with carbonic anhydrase I and II (about 40% homology with both of them at the identical amino acid sequence level). We think this sequence corresponds to a new type of carbonic anhydrase that is specifically expressed in cerebellum. Although physiological roles for each type of carbonic anhydrase are not well understood, interesting variations in tissue- and cell-type distributions of the isozymes are known. At present it is not certain whether the *nervous* mutation affects directly or indirectly the expression of the gene isolated.

Myofibril Assembly is linked with Vinculin, α-Actinin and Cell-Substrate Contacts in Cardiac Myocytes *in vitro*

Yutaka SHIMADA

The relationship of nascent myofibrils with the accumulation of adhesion plaque proteins and the formation of focal cell contacts was studied in embryonic chick cardiac myocytes *in vitro*. The cultures were double-stained with various combinations of the specific antiactin drug phalloidin and antibodies against vinculin, α-actinin, connectin (titin), myosin heavy chain, fibronectin and desmin, and examined under fluorescence and interference reflection microscopy.

Filament bundles with and without cross-striations in cardiac myocytes were reactive to antibodies against muscle-specific proteins and, thus were regarded as nascent myofibrils. The distribution pattern of these fibrillar structures was generally in correlation with the shape of the cells. They were aligned parallel to the long axis of the cells within their straight parts. In polygonal areas, they were prominent as spanning cellular diagonals but

some were also observed along perimeters. Both ends of the nascent myofibrils terminated at areas where vinculin and α-actinin, proteins thought to be involved in the attachment of actin to the membrane, were concentrated. These areas overlapped with dark images seen with the interference reflection. Fibronectin was located coincidentally in proximity to these dark areas.

These results indicate that the formation of focal contacts and the accumulation of adhesion plaque proteins at these sites are prerequisite to or, at least, somehow related with the polymerization and regular alignment of sarcomeric proteins. This suggests that the distribution of these protein molecules must be polarized along lines that are generated between the sites of focal cell contacts and, consequently, myofibrillogenesis occurs along these inferred lines. Additionally, connectin and desmin do not appear to form the initial scaffold for sarcomeres.

Myogenesis *in vitro* as Seen with the Scanning Electron Microscope

Yutaka SHIMADA

We describe in this paper our recent observations by scanning electron microscopy (SEM) on the differentiation of cell surface and cytoplasmic organelles in embryonic chick skeletal muscle cells *in vitro*. The changes in surface structures of myoblasts during mitosis were essentially similar to those of other cell types, but the characteristic spindle shape of myoblasts did not change throughout most of this period. Cytoskeletal structures under the sarcolemma were examined by Triton extraction and metal coating. Cells in S, G_2 and M possessed a dense filament network, and those in G_1 a loose one under the membrane. Myotubes possessed a dense network under the sarcolemma. In the fusion area between a myoblast and a myotube, the cytoskeletal domain of the former could be distinguished from the latter because of the mosaic appearance of the subsarcolemmal cytoskeletal network. This network was composed predominantly of 10–13 nm filaments; they were identified as actin filaments because of their decoration with myosin subfragment-1. Triton treatment and thiocarbohydrazide-osmium staining allowed us to visualize myofibrils. They ran in the direction of inferred stress lines brought about by elongation and adhesion of the cells to the substrate. Intracellular membranous organelles could

be seen by the freeze-polishing and osmium-maceration procedure. Mitochondria exhibited complex irregular branchings with T system tubules running a tortuous course. Sarcoplasmic reticula with occasional dilatations were found to be connected to each other. These results are of sufficient promise to encourage more extensive analysis of myogenesis by SEM.

V. CYTOGENETICS

Expression of Ribosomal RNA Gene Clusters in Laboratory Strains of Mice Demonstrated by Cytogenetic Techniques

Yasuyuki KURIHARA, Dong Sang SUH, Hitoshi SUZUKI* and Kazuo MORIWAKI

Several cytogenetic techniques can be applied to detect ribosomal RNA gene (rDNA) clusters on chromosome preparations. The most commonly used method is Ag-NOR staining which is specific for transcriptionally active rDNA clusters. Chromosome location of rDNA clusters can be also demonstrated directly by *in situ hybridization*, irrespective of gene expression. By combining these two techniques, therefore, it is possible to distinguish transcriptionally active rDNA from inactive ones. In the present study, we examined chromosome locations and the expression of rDNA clusters in twelve laboratory strains of mice.

For chromosome preparations, short term cultures of spleen cells were used. After G-banding for chromosome identification, each preparation

Table 1. Chromosome locations of ribosome RNA gene clusters demonstrated by Ag-NOR staining and *in situ hybridization*

Strain	Ag-NOR staining					*in situ* hybridization				
	12	15	16	18	19	12	15	16	18	19
AKR/J	+	+		+		+	+	+	+	+
BALB/cAnN	+	+		+		+	+	+	+	+
BALB/cHeA	+	+		+		+	+	+	+	+
BALB/cJ	+	+	+	+		+	+	+	+	+
BALB/cUCSD	+	+		+		+	+	+	+	+
C57BL/10SnJ	+	+			+	+	+		+	+
C57BL/6J	+	+	+	+	+	+	+	+	+	+
CBA/CaHN		+	+	+			+	+	+	
CBA/StMs		+		+	+		+	+	+	+
SM/J			+	+		+		+	+	+
SWR/J	+	+		+	+	+	+		+	+
129/J	+		+	+	+	+		+	+	+

* Jikei Medical School.

was subjected to either Ag-NOR staining or *in situ hybridization.* Results are shown in Table 1. Chromosome locations of rDNA clusters are closer to the centromere on chromosomes 12, 15, 16, 18, and 19. Moreover seven out of twelve strains have one or two inactive rDNA clusters, in addition to an active one. The same activity patterns observed in different individuals of the same strains, suggest this is a fixed genetic character. Tissue specificity of expression was also examined in bone marrow cells and meiotic cells from CBA/J male mice, The rDNA clusters on chromosome 16 were not stained by Ag-NOR staining in all preparations. The mechanism of inactivation for the rDNA clusters as a genetic trait remain under study.

Chromosomal Polymorphism in the Ant *Myrmecia* (*pilosula*) *n=1*

Hirotami T. IMAI, Robert W. TAYLOR*, Ross H. CROZIER**,
Michael W. L. CROSLAND** and Graeme P. BROWNING***

Ants of the species group, *Myrmecia pilosula* (Fr. Smith), are commonly referred to in Australia as 'Jack-jumpers', and are among the most primitive of all living formicids. Their chromosome numbers were first reported to include 2n=9, 10, 31–32, by Imai *et al.* (1977, *Chromosoma*, **59**: 341–393). At that time only one species was recognized in taxonomic literature. More recently a 'pilosula' colony collected at Tidbinbilla near Canberra, and reported by Crossland and Crozier (1986, *Science* **231**: 1278), has yielded the lowest chromosome number known among higher organisms (or indeed possible), namely n=1 and 2n=2.

Encouraged by these findings we have established an international co-operative programme aimed at the karyological and taxonomic survey of the genus *Myrmecia*, especially, at this stage, of the *M. pilosula* group, the species now referred to collectively as *Myrmecia* (*pilosula*). Large-scale field studies were carried out in 1985 and 1987, supported by the Overseas Visitors Programme of the CSIRO Division of Entomology (Australia), and by a Grant-in-Aid for Overseas Scientific Research from the Japanese Ministry of Education. During these surveys more than 150 *M.* (*pilosula*)

* Division of Entomology, C.S.I.R.O., Canberra.
** School of Biological Science, The University of New South Wales, Sydney.
*** Department of Zoology, University of Adelaide.

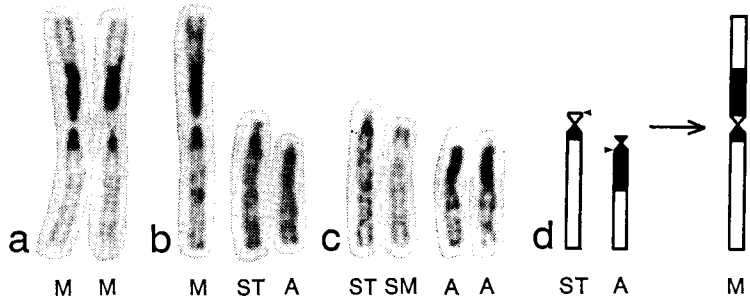


Fig. 1. Polymorphic karyotypes of *Myrmecia* (*pilosula*) $n=1$. a: 2n=2. b: 2n=3. c: 2n=4. d: A chromosomal rearrangement (tandem fusion) involved in this polymorphism. Darkly stained regions indicate C-banding. Arrow heads indicate the presumed breakage points.

colonies were collected in New South Wales, the Australian Capital Territory, Victoria, South Australia and Tasmania. Species of the group were found to be karyologically highly differentiated. The range of chromosome numbers observed was 2n=2, 3, 4, 9, 10, 15, 17–32. Significant but subtle morphological divergence has also been observed, often involving characteristics previously accorded little taxonomic importance. This has improved taxonomic acuity for revisionary studies on *Myrmecia* at large, which probably includes over 200 Australian species (only about half of which have scientific names), and one from New Caledonia.

It is now clear that the *M.* (*pilosula*) complex includes at least 5 separate, similar biological species. Details will be published elsewhere. We provide here a brief summary of chromosomal observations on the species currently denoted *M.* (*pilosula*) $n=1$, which was mentioned above.

M. (*pilosula*) $n=1$ is distinguished from other members of its group by its stocky form and details of cuticular sculpture and pilosity. It builds relatively small nest mounds compared to most other species. A total of 11 colonies (with the code numbers indicated) have been collected from Tidbinbilla, A.C.T. (HI87-151, 157), Canberra City, A.C.T. (HI87-165, 235), the Mongarlowe/Charleyong district, N.S.W. (HI87-136, 148, 150, 151, 153, 154, 157) and South Warrandyte, Vic. (HI87-213).

We have found that this species is in fact chromosomally polymorphic, with 2n=2, 3 or 4. Five karyologically different colony series have been discriminated, as follows: 2n=2 (HI87-151, 157); 2n=2/3 (HI87-148, 150,

153, 213); 2n=3/4 (HI87-136, 154); 2n=4 (HI87-165); and 2n=2/3/4 (HI87-235). In the last colony the three karyotypes formulated are 2K=2M (Fig. 1a), 2K=1M+1ST+1A (Fig. 1b), and 2K=1SM+1ST+2A (Fig. 1c) respectively. The C-banding patterns suggest a strong homology between the long arm of the large M of 2n=2 cells and ST of 2n=4 cells, and likewise between the short arm of M and A of the latter. This indicates that the large M chromosome probably originated secondarily following a tandem fusion which occurred between the short arm terminal of ST and a proximal region of the long arm of chromosome A (Fig. 1d).

A Note on Trophic Egg-feeding by the Queen of the Primitive Ant *Myrmecia* (*pilosula*) *n=1*

Keiichi MASUKO

The early history of social evolution in ants is obscure. This is due to the lack of an adequate fossil record of possible wasp-like ancestors for the fully-eusocial formicids, and because no living non-eusocial sister group is known. Studies of the social behaviour of primitive species in genera such as *Amblyopone* and *Myrmecia* can, however, assist in the reconstruction of possible evolutionary events. The feeding behaviour of the queen is a key attribute for such an approach. It is well known that *Myrmecia* workers lay non-reproductive eggs which are passed as food to the colony queen, and to larvae and other workers. These are called 'trophic eggs'.

By observing a mature laboratory colony of *Myrmecia* (*pilosula*) *n=1* I have sought to understand how the queen obtains trophic eggs from donor workers, and how the eggs contribute to her nutrition. Despite the continuous accessibility of captured insect remains in the nest, the queen was not observed to feed upon prey. She fed only on worker-laid trophic eggs, which were consumed at the rate of 1.0 ± 0.9 (SD) per hour. When soliciting a trophic egg the queen always approached a potential donor worker and stroked its body strongly with her antennae, after which the worker sometimes laid a trophic egg. This response by workers was not always positive, because most moved away from the queen when solicited, or remained still and unresponsive until the queen ceased stroking and moved away herself. Thus, the queen obtained eggs for only 7.5% of all solicitations. The queen was never observed to obtain eggs without soliciting. Oral regurgitative feeding between workers and the queen (which has

been noted in other *Myrmecia* species (R. W. Taylor pers. com.)) was not seen. The queens in mature colonies of *M.* (*pilosula*) *n=1* are thus apparently completely dependent upon worker-laid trophic eggs for their nutrition.

VI. MUTAGENESIS AND RADIATION GENETICS

Inactivation of Transforming DNA with Gamma-rays at Low Dose Rates

Yoshito SADAIE and Tsuneo KADA

As tritiated water inactivates bacterial transforming DNA more efficiently at lower concentrations of the tritium atom, a similar effect was hypothesized for gamma-rays at lower dose rates. Transforming DNA from the *Bacillus subtilis* wild type strain was dissolved in $\times 0.1$ SSC (standard saline citrate) at a concentration of 5 μg/m*l*. The sample was irradiated with gamma-rays at room temperature from ^{137}Cs (33kR/h–0.2KR/h) and ^{60}Co (40R/h–0.2R/h) and the remaining Arg^+ transforming activity was examined with the arginine requiring *B. subtilis* mutant strain. The LD_{37} obtained varied from 111R (33KR/h) to 300R (0.2R/h), suggesting no dose rate effect for DNA inactivation with gamma-rays.

Tissue-specific Radiation Sensitivity and Its Relation to Differentiation in a Mouse Mutant, Wasted

Hideo TEZUKA and Tadashi INOUE

Tissue specificity on radiation sensitivity was investigated in the mouse mutant, wasted. The two endpoints used were the induced frequency of chromosomal aberrations examined 18 or 24 h after gamma-ray-irradiation and changes in survival of cultured cells.

Studies for the former consisted of *in vivo*- and *in vitro*-irradiation treatments: *in vivo*, bone marrow and spermatogonia; *in vitro*, erythropoietin-stimulated bone marrow cells and fibroblasts in culture. Radiation sensitivity was observed in bone marrow cells in both in vivo and in vitro cases. No differences in sensitivity were shown in the other cases between wasted and littermate control mice. Studies for the latter used bone marrow cells in culture, and revealed that differentiated erythropoietic stem cell, CFU-E, was sensitive, while the granulopoietic stem cell, CFU-C, was not sensitive.

These results suggest that the effect of the *wst* gene is tissue- and even cell-specific in the wasted mouse and that radiation sensitivity in the bone marrow may be based on changes in the erythropoietic cell lineage itself.

Mutagen-Modifying Effects of Vitamin A and Vitamin E in Cultured Chinese Hamster Cells

Yukiaki KURODA

Most mutagens present in our environment are modified in their mutagenic activity by other substances in their presence. In the previous report, it was found that vitamin C and its derivatives reduced the cytotoxicity and mutagenicity of ethyl methanesulfonate (EMS) in Chinese hamster cells. In the present study, vitamin E which is an antioxidant and has a stabilizing effect on cell membrane, and vitamin A, which shows various physiological activities in living organisms, were examined for their activity modifying the mutagenicity of EMS.

Cells used were a clonal line of Chinese hamster V79 cells. Mutations induced by EMS were detected by using the 6-thioguanine (6TG)-resistant marker. Cells were treated with EMS alone or together with vitamins for 3 hours. The cytotoxicities and induced mutation frequencies were determined by the colony-forming activity of treated cells in normal medium or in 6TG-containing medium. EMS alone had a cytotoxic effect on the cells, showing an LD_{50} of 540 μg/m*l*. Vitamin E had a moderate cytotoxicity, with the LD_{50} of 710 μg/m*l*. In the presence of vitamin E, the cytotoxicity of EMS increased, and showed a greater value than the sum of cytotoxicities of both substances. Vitamin A had a strong cytotoxic effect, showing an LD_{50} of 302 μg/m*l*. In the presence of vitamin A at a concentration of 100 μg/m*l*, which had no cytotoxicity, the cytotoxicity of EMS was slightly reduced.

EMS alone showed a strong activity in inducing 6TG-resistant mutations in V79 cells. At a concentration of 1,000 μg/m*l*, EMS induced 6TG-resistant mutations at a frequency of 88×10^{-5}. Vitamin E alone had no mutagenic effect on the cells. However, in the presence of vitamin E, the EMS-induced mutations increased markedly, depending on concentrations of vitamin E. Vitamin E at a concentration of 100 μg/m*l* enhanced the frequency of EMS-induced mutations three times.

This enhancing effect of vitamin E was detected only when vitamin E coexisted with EMS: a pretreatment with vitamin E had no effect on EMS-induced mutations. Since vitamin E with an antioxidant activity was not antimutagenic, it suggests that the antimutagenicity of vitamin C may be due to some mechanism other than antioxidant activity.

Vitamin A alone also had no mutagenic effect on cells at concentrations up to 100 μg/ml. In the presence of vitamin A, the EMS-induced mutations reduced to 1/2–2/3, depending on the dosage of the vitamin A (200–600 μg/ml).

Mutagenicity of N^4-Aminocytidine and Its Derivatives in Chinese Hamster V79 Cells

Yukiaki KURODA, Akinori NOMURA*, Kazuo NEGISHI* and Hikoya HAYATSU*

Most mutagens are DNA-attacking agents causing damage in the structure of the nucleic acid, leading to the production of mutated progenies through the process of DNA replication. On the other hand, pyrimidine and purine bases structurally different from DNA bases can be mutagenic by incorporating into DNA and causing mutations due to their erroneous base-pairing properties. In the present study, DNA-precursor type mutagens, N^4-aminocytidine and its derivatives were tested for their mutagenicity in cultured Chinese hamster V79 cells.

N^4-aminocytidine and N'-methy-N^4-aminocytidine showed a strong mutagenicity in inducing 6-thioguanine (6TG)-resistant mutations. N'-(2-hydroxyethyl)-N^4-aminocytidine, which had an alkyl group on the N'-nitrogen of the hydrazino group at position 4 of N^4-aminocytidine also had a strong mutagenic activity. On the other hand, N^4-aminocytidine derivatives with an alkyl group on the N^4-nitrogen and N^4-aminodeoxycitidine were not mutagenic for V79 cells.

To investigate the mechanism of the strong mutagenic activity of N^4-aminocytidine, the incorporation of H^3-labeled N^4-aminocytidine into DNA bases was examined. As a result of this experiment, the H^3-labled compound was incorporated into a N^4-aminodeoxycitidine fraction. This suggests that N^4-aminocytidine may be de-hydrozinated to form uridine, followed by metabolic conversion of the uridine into deoxycitidine triphosphate.

We previously found that N^4-aminocytidine incorporated into DNA produced base-pair transitions, i.e., TA to CG and CG to TA in phage and bacteria. It is likely that this type of mutation is also taking place in V79 cells. For details, see *Mutation Research* **177**: 283–287 (1987).

* Faculty of Pharmacentical Sciences, Okayama University, Okayama.

Major Genetic Loci Regulating Susceptibility to Pulmonary Adenomas in CXB Recombinant Inbred Strains of Mice

Nobumoto MIYASHITA and Kazuo MORIWAKI

To determine the major genetic loci regulating susceptibility to pulmonary adenomas, CXB recombinant inbred (RI) strains of mice, A/Wy and their F1 progenies received a single s.c. injection of 1.5 mg urethane/g body weight. These mice were sacrificed in the 5th month after treatment. After the lungs were fixed in ethanol/formaldehyde (9: 1), the number of adenoma foci was assessed. The progenitor strains, BALB/c and C57BL/6, and 7 recombinants could be divided into two groups with respect to the

Table 1. Urethane-induced pulmonary adenomas in CXB recombinant inbred, A/Wy and their F1 hybrids

CXB strain	No. of tumors (mean±SEM)	Incidence: No. of mice with tumors/total no. of surviving mice
BALB/c	6.76±0.81	21/21
B6	0.53±0.17	7/17 (41%)
CXBG	18.17±2.07	6/ 6
CXBH	10.29±1.60	7/ 7
CXBE	9.33±1.02	15/15
CXBJ	7.80±0.88	15/15
CXBD	1.30±0.35	7/10 (70%)
CXBK	0.86±0.34	4/ 7 (57%)
CXBI	0.50±0.29	2/ 4 (50%)
F1 hybrids between A/Wy and CXB RI strains	**No. of tumors (mean±SEM)**	**Incidence: No. of mice with tumors/total no. of surviving mice**
A/WySnJ	27.15±1.02	46/46
(A×BALB/c)F1	18.42±1.85	7/ 7
(A×B6)F1	18.71±0.81	31/31
(A×CXBG)F1	24.60±1.24	10/10
(A×CXBH)F1	17.80±1.07	30/30
(A×CXBE)F1	15.30±1.75	10/10
(A×CXBJ)F1	16.64±1.73	11/11
(A×CXBD)F1	24.86±1.08	14/14
(A×CXBK)F1	18.40±1.40	5/ 5
(A×CXBI)F1	17.29±1.22	17/17

average number of adenoma foci per mouse: one labeled susceptible, to which strains BALB/c, CXBE, CXBG, CXBH and CXBJ belong, and the other resistant, which included strains CXBD, CXBK, CXBI and C57BL/6 (Table 1). The average number of adenoma foci of the former group and the latter one ranged from 6.76 (BALB/c) to 18.17 (CXBG) and from 0.50 (CXBI) to 1.30 (CXBD), respectively. The strain distribution pattern of adenoma susceptibility were exactly the same as that of the locus at major urinary protein-1 (*Mup*-1) loci on chromosome 4 in CXB RI strains. Minor differences in susceptibility were found in each group. The average number of adenoma foci in CXBG and CXBD is twice that of the other 4 susceptible and 3 resistant strains, respectively. In (A×B6)F1 and (A×BALB/c)F1, intermediate responses were observed. The number of adenoma foci in F1 hybrids between strain A and the CXB strains were also intermediate except for (A×CXBG)F1 and (A×CXBD)F1, which showed susceptible phenotypes like A/Wy strains. From these results, it can be argued that one major gene responsible for adenoma susceptibility is located near *Mup*-1 locus on chromosome 4. In addition, other minor genes were also found to exist, but not located near *Mup*-1.

Genetic Analysis of the Reciprocal Translocation between a Sex Chromosome (Y) and an Autosome (5) in the Silkworm

Akio MURAKAMI

Many translocation chromosomes have been detected, and research has been carried out chromosome markers containing the visible character gene on sex chromosome (Y). Therefore, few approaches have been made to study the mechanism of the production of translocation chromosomes, and there has only been an analysis of a strain with reciprocal translocations of the zebra gene on the Y chromosome and an autosome (3) (Hashimoto, 1957). He reported that most of the visible character genes were attached to the translocated chromosome.

We treated female pupae (oocytes) labeled by the Y translocation chromosome with ethyl nitrosoguanidine, and mated them with males with an autosome (5) labeled by *pe* and *re* egg color genes immediately after emergence. We detected mosaics with black egg color and yellowish white color (*pe*), which can be used to estimate the mutation in the *pe* locus of the

egg. The mutant egg was subjected to the progeny test.

Males and females were found to derive from black and white eggs, respectively. The viability (hatchability) of the black eggs tended to be significantly higher than that of the yellowish white eggs. The percentage of *zebra* larvae was the same as that of plain larvae for both females and males. A female (or male) *zebra* mutant with normal epidermis was mated with a male (or female) labeled with the recessive *pe*, *ok* or *re* gene, and the characteristics of the next generation were analyzed. Females were derived from black eggs and males from yellow eggs, respectively. The *ok* character was observed in all male larvae, but not in any female larvae. Since no red eggs appeared in the males or females, judging from the present mating scheme, the fragment of the 5th autosome translocated on the Y chromosome was found to contain the ok^+ (4.7) locus as well as the pe^+ (0.0) locus. Male larvae in the yellowish white eggs of the present translocated strain were crossed with females of the *pe*: *re* marker stock. About 25% of the eggs (embryos) obtained by mating among the *zebra* larvae were dead.

Therefore, this strain was confirmed to be deficient in the autosome (5). At the same time, the *zebra* part fragmented from the Y^{ze} translocation chromosome was found to be translocated to the deficient part. That is, in the present translocation chromosome induced by a chemical mutagen, we concluded that fragmentation occurred simultaneously in the Y chromosome and the autosome (5), and the fragments showed reciprocal translocation with mutual translocation of the fragmented part.

VII. POPULATION GENETICS

Molecular Evolutionary Clock and the Neutral Theory

Motoo KIMURA

From the standpoint of the neutral theory of molecular evolution, it is expected that a universally valid and exact molecular evolutionary clock would exist if, for a given molecule, the mutation rate for neutral alleles per year were exactly equal among all organisms at all times. Any deviation from the equality of neutral mutation rate per year makes the molecular clock less exact. Such deviation may be due to two causes: one is a change in mutation rate per year (such as due to change in generation span), and the other is the alteration of the selective constraint for each molecule (due to change in the internal molecular environment). A statistical method was developed to investigate the equality of evolutionary rates among lineages. This was used to analyze protein data to demonstrate that these two causes are actually at work in molecular evolution. It was emphasized that departures from exact clockwise progression of molecular evolution by no means invalidates the neutral theory. It was pointed out that experimental studies should be undertaken to settle the issue of whether the mutation rate for nucleotide change is more constant per year or per generation among organisms whose generation spans are very different. For details, see *J. Mol. Evol.* **26**: 24–33 (1987).

A Stochastic Model of Compensatory Neutral Evolution

Motoo KIMURA

Using the diffusion equation method, I investigated the average time until fixation of a mutant gene or genes in a finite population under continued (irreversible) mutation pressure. I mainly considered the situation in which the initial population consists exclusively of the wild type allele (or alleles). I denote by v the mutation rate per locus per generation.

The treatment for the single locus case (with a pair of alleles A and A') is straightforward. It shows that for a slightly deleterious mutant the average time taken for fixation is too long to be of practical significance in

evolution unless $4N_e s' < 10$, where N_e is the effective population size and s' is the selection coefficient against the mutant allele (A').

The main aim of this paper is to investigate the two-locus cases. I assumed a pair of alleles A and A' at the first locus, and B and B' at the second locus, and investigated the situation in which mutations are individually deleterious but become harmless (i.e., selectively neutral) in combination. Such mutations may be termed 'compensatory neutral mutations.' I assigned relative fitnesses 1, $1-s'$, $1-s'$ and 1 respectively to AB, $A'B$, AB' and $A'B'$. Two extreme cases, i.e., (i) free recombination, and, (ii) complete linkage between the loci were considered. Assuming $2N_e v = 1$, where v is the mutation rate per locus, the following results were obtained. (i) In the case of free recombination, the average time until fixation ($\bar{T}$) was about $5N_e$ generations for neutral mutations ($s' = 0$). For slightly deleterious mutations in the range $0 < 4N_e s' \leqq 10$, $\bar{T}$ was slightly shorter but not very much (e.g. $\bar{T} = 4.5N_e$ for $4N_e s' = 10$). If $4N_e s'$ was much larger, the fixation time quickly became very large, and for mutations with $4N_e s' > 40$, $\bar{T}$ became so enormously large that such mutations are unlikely to play a part in evolution. (ii) In the case of complete linkage, single mutations with much larger deleterious effects are allowed to participate in "compensatory neutral evolution," namely, joint fixation of the selectively neutral double mutant ($A'B'$) occurs without having to wait an unrealistically long time. In fact, even for $4N_e s' = 400$, the average fixation time was only 10 times as long as the completely neutral case. The bearing of this finding on molecular evolution was discussed with special reference to coupled substitutions at interacting amino acid (or nucleotide) sites within a folded protein (or RNA) molecule. It was concluded that compensatory neutral mutations may play an important role in molecular evolution. For details, see in *Lecture Notes in Biomathematics* Vol. 70, pp. 2–18 (1987).

Very Slightly Deleterious Mutations and the Molecular Clock

Tomoko OHTA

A model for very slightly deleterious mutations was examined from the standpoint of population genetics in relation to the molecular evolutionary clock. The distribution of selection coefficients of mutants (in terms of amino acid changes) with little effect is thought to be continuous around zero, with an average negative value. The variance in selection coefficients

depends upon environmental diversity and hence on total population size of a species. By considering various examples of amino acid substitutions, the average and standard error of selection coefficients and the reciprocal of population size are assumed to have similar values. The model predicts a negative correlation between evolutionary rate and population size. This effect is expected to be partially cancelled with the generation time effect in intrinsic mutation rate. Implications of this prediction on the molecular evolutionary clock were discussed. For details, see *J. Mol. Evol.* **26**: 1–6 (1987).

A Model of Evolution for Accumulating Genetic Information

Tomoko OHTA

By taking into account recent knowledge of multigene families and other repetitive DNA sequences, a model for evolution by gene duplication for accumulating genetic information was studied. Genetic information is defined as the sum of distinct functions that the gene family can perform. A coefficient, "genetic diversity" defined and used in this study, was highly correlated with genetic information. Initially, a multigene family with a few gene copies was assumed, and natural selection started to work on this gene family to increase genetic diversity contained in the gene family. As an important mechanism, unequal crossing-over was incorporated. Together with mutation, it was responsible for supplying genetic variability among individuals for selection to work. A specific model, in which individuals with less genetic diversity are selectively disadvantageous, has been studied in detail. Through approximate theoretical analysis and extensive Monte Carlo studies, it has been shown that the system is an extremely efficient way to accumulate genetic information. For attaining one gene, the genetic load is much smaller under this model than under the traditional model of natural selection. The model may be applied to the process of origin of multigene families with diverse copy members such as those of immunoglobulin or cytochrome P450. In general, the process of creating new genes by duplication might be somewhere between the present and the traditional models. For details, see *J. Theor. Biol.* **124**: 199–211 (1987).

Simulating Evolution by Gene Duplication

Tomoko OHTA

By considering the recent finding that unequal crossing-over and other molecular interactions are contributing to the evolution of multigene families, a model of the origin of repetitive genes was studied using Monte Carlo simulations. Starting from a single gene copy, how genetic systems evolve was examined under unequal crossing-over, random drift and natural selection. Both beneficial and deteriorating mutations were incorporated, and the latter were assumed to occur ten times more frequently than the former. Positive natural selection favors those chromosomes with more beneficial mutations in redundant copies than others in the population, but accumulation of deteriorating mutations (pseudogenes) have no effect on fitness so long as there remains a functional gene. These results imply the following: (1) Positive natural selection was needed in order to acquire gene families with new functions. Without it, too many pseudogenes accumulated before attaining a functional gene family. (2) There was a large fluctuation in the outcome even if parameters were the same. (3) When unequal crossing-over occurred more frequently, the system evolved more rapidly. It was also shown, under realistic values of parameters, that the genetic load for acquiring a new gene is not as large as J. B. S. Haldane suggested, but not so small as in a model in which a system for selection started from already redundant genes. For details, see *Genetics* **115**: 207–213.

On the Overdispersed Molecular Clock

Naoyuki TAKAHATA

Rates of molecular evolution at some loci are more irregular than described by simple Poisson processes. Three situations under which molecular evolution would not follow simple Poisson processes were reevaluated from the viewpoint of the neutrality hypothesis: (i) concomitant or multiple substitutions in a gene, (ii) fluctuating substitution rates in time caused by coupled effects of deleterious mutations and bottlenecks, and (iii) changes in the degree of selective constraints against a gene (neutral space) caused by successive substitutions. The common underlying assumption that these causes are lineage nonspecific excludes the case where mutation rates

themselves change systematically among lineages or taxonomic groups, and severely limits the extent of variation in the number of substitutions among lineages. Even under this stringent condition, however, the third hypothesis, the fluctuating neutral space model, can generate fairly large variations. This was described by a time-dependent renewal process, which did not exhibit any episodic nature of molecular evolution. It was argued that the observed elevated variances in the number of nucleotide or amino acid substitutions do not immediately call for positive Darwinian selection in molecular evolution. For details, see *Genetics* **116**: 169–179, 1987.

Adult Lactose Absorption and Milk Use from the Standpoint of Gene-culture Theory

Kenichi AOKI

Two major hypotheses proposed to explain the observed association between prevalence of adult lactose absorption and milk use in human populations are the "culture historical hypothesis" and the "calcium absorption hypothesis". A rigorous formulation of the evolutionary problem calls for an approach based on gene-culture theory. This work selectively reviewed data bearing on the coevolutionary approach. First, a reinterpretation of the data on Finnish school children suggested that lactose malabsorption is fully expressed by age 12 rather than after age 15. This result was consistent with a one-locus two-allele determination of the trait, and also suggested that lactase is not an inducible enzyme. Second, a comparison of milk preferences in lactose absorbers and malabsorbers suggested that a difference in preference may manifest itself when malabsorbers are in the majority, as was presumably the case in ancestral human populations. Third, calcium absorption in the presence of lactose is more efficient in lactose absorbers than malabsorbers. This result supported the contention that malabsorbers were more susceptible to bone diseases and hence at a selective disadvantage in northern Europe. Fourth, a simple model of gene-culture coevolution was presented to show how the reviewed data pertain. The time required for genetic change was computed as a function of the intensity of selection. Strong selection pressures must be invoked if the genetic change is to have occurred by natural selection during the 6000 years since adoption of milk use. Finally, the incompleteness of the association between prevalence of adult lactose absorption and milk use

among the populations surveyed was discussed. For details, see *Jpn. J. Genet.* **62**: 445–459.

Toward a Theory for the Evolution of Cultural Communication: Coevolution of Signal Transmission and Reception

Kenichi AOKI and Marcus W. FELDMAN

A haploid sexual two-locus model of gene-culture coevolution was examined, in which a dichotomous phenotype subject to natural selection is transmitted vertically with probabilities dependent on the chosen parent's genotype and phenotype and the offspring's genotype. Stability conditions for the genetically monomorphic corner equilibria were obtained. In a specialization of this general model, one locus controls the transmission and the other controls the reception of adaptive information. The corner and edge equilibria of this doubly coevolutionary model were fully analyzed, and conditions for transmission and reception to coevolve were derived in terms of the efficiency of vertical transmission, the selective advantage gained from possessing the information, the costs of transmission and reception, and the recombination fraction between the two loci. Possible applications of the model are to the evolution of semantic alarm calls in vervet monkeys and the phonetic aspects of human language. In a third model with diploid genetics, we considered the initial increase in cultural transmission from a mutation-selection balance in which the adaptive phenotype is the consequence of a dominant gene at one locus. A second gene controls the transmission of the phenotype in such a way that a new mutant at this second locus permits learning of the adaptive phenotype from a parent who has it. This new mutant cannot increase when rare. For details, see *Proc. Natl. Acad. Sci. USA* **84**: 7164–7168.

Gene-culture Waves of Advance

Kenichi AOKI

Major genetic and cultural changes may have been coupled during hominid evolution. Since hominids have had a wide geographical distribution for about one million years, any mutant gene or cultural innovation that became established had to spread from its origin. A pair of nonlinear diffusion equations was derived which models the propagation

of a mutant gene and a cultural innovation. Both were assumed to originate in the same locality along a linear habitat. The mutant gene and its allele are semidominant, and the two cultural choices are transmitted according to what I call the logistic attraction-repulsion model. The genes influence cultural choice, and the two interact to determine fitness. Of particular interest is the case in which mutant gene and cultural innovation are mutually dependent, neither being able to spread without the other. Each equation of the pair is similar in form to Fisher's equation, with a linear function of the other dependent variable replacing the constant coefficient in the reaction term. The partial differential equations were solved numerically to obtain the asymptotic speeds. Their form also suggested an heuristic argument which proved useful, but I was unable to obtain any analytic results. The waves of the system were shown to be of two types, synchronous and asynchronous. When genes and culture are mutually dependent, synchronous traveling waves can exist. However, their existence is dependent on initial conditions, and the speed of propagation is slow. For details, see *J. Math. Biol.* **25**: 453–464.

VIII. EVOLUTIONARY GENETICS

Molecular Evolutionary Rates of Oncogenes

Takashi GOJOBORI and Shozo YOKOYAMA

Using nine sets of viral and cellular oncogenes, the rates of nucleotide substitutions were computed using Gojobori and Yokoyama's (1985) method. The results obtained confirmed our previous conclusion that the rates of nucleotide substitution for viral oncogenes are about a million times higher than those for their cellular counterparts. For cellular oncogenes and most viral oncogenes, however, the rate of synonymous substitution is higher than that of nonsynonymous substitution. Moreover, the pattern of nucleotide substitutions for viral oncogenes is more similar to that for functional genes (such as cellular oncogenes) than for pseudogenes. This implies that nucleotide substitutions in viral oncogenes may be functionally constrained. Thus, our observations of nucleotide substitutions for oncogenes in those DNA and RNA genomes are consistent with Kimura's neutral theory of molecular evolution (Kimura 1968, 1983). For details, see *J. Mol. Evol.* **26**: 148–156, and *Jpn. J. Genet.* **62**: 163–177.

Organization and Nucleotide Sequence of a Densovirus Genome Imply a Host-Dependent Evolution of the Parvoviruses

Hisanori BANDO, Jun KUSUDA, Takashi GOJOBORI
Takeo MARUYAMA and Shigemi KAWASE

The genome structure of a densovirus in silkworm was determined by sequencing more than 85% of the complete genome DNA. The genome organization of an insect parvovirus was deduced from the DNA sequence. In the viral genome, two large open reading frames designated 1 and 2 and one smaller open reading frame designated 3 were identified. The first two open reading frames shared the same strand, while the third was found in the complementary sequence. Computer analysis suggested that open reading frame 2 may encode all four structural proteins. The genome organization and a part of the nucleotide sequence were conserved among

the insect densovirus, rodent parvoviruses, and a human dependovirus. These viruses may have diverged from a common ancestor. For details, see *J. Virol.* **61**: 553–560.

Evolutionary Origin of Pathogenic Determinants in Enterotoxigenic *Escherichia coli* and *Vibrio cholerae* 01

Tatsuo YAMAMOTO, Takashi GOJOBORI and Takeshi YOKOTA

Three families of the pathogenic determinants in enterotoxigenic *Escherichia coli* and *Vibrio cholerae* 01 are evolutionarily related to each other. These three families are (1) a family of cholera enterotoxin (CT) and heat-labile enterotoxin (LT) including CT, LTh, and LTp, (2) a family of heat-stable enterotoxin I (STI) including STIa and STIb, and (3) a family of K88 enteroadhesion fimbriae including K88ab, K88ac, and K88ad. They were analyzed for synonymous (silent) nucleotide substitutions, using gene nucleotide sequences of earlier reports and a newly determined LTp gene nucleotide sequence. The results obtained suggest that the divergences between LT and CT and between STIa and STIb occurred in the remote past, whereas those between LTh and LTp and between members of the K88 family occurred very recently. We therefore concluded that the LT gene is a foreign gene that has been acquired by *E. coli* to form an enteropathogen. This provides evolutionary evidence of species-to-species transfer of pathogenic determinants in prokaryotes. For details, see *J. Bact.* **169**: 1352–1357.

Molecular Evolution and Phylogeny of the Human AIDS Viruses LAV, HTLV-III, and ARV

Shozo YOKOYAMA and Takashi GOJOBORI

A phylogenetic tree for human lymphadenopathy-associated virus (LAV), human T-cell lymphotrophic virus type III (HTLV-III), and acquired immune deficiency syndrome (AIDS)-associated retrovirus (ARV) has been constructed from comparisons of the amino acid sequences of their *gag* proteins. A method was proposed for estimating the divergence times among these AIDS viruses and the rates of nucleotide substitution for their RNA genomes. Analyses indicate that the LAV and HTLV-III strains

diverged from one another after 1977 and that their common ancestor diverged from the ARV virus no more than 10 years earlier. Hence, the evolutionary diversity among strains of AIDS viruses apparently has been generated within the last 20 years. It is estimated that the genome of the AIDS virus has a nucleotide substitution rate on the order of 10^{-3} per site per year, with the rate in the second half of the genome being double that in the first half. For details, see *J. Mol. Evol.* **24**: 330–336.

Molecular Phylogeny of the Human Immunodeficiency and Related Retroviruses

Shozo YOKOYAMA, Etsuko N. MORIYAMA and Takashi GOJOBORI

Human immunodeficiency virus (HIV) is the aetiological agent of acquired immune deficiency syndrome (AIDS). It is known that HIVs are more closely related to lentiviruses than to oncoviruses (Sonigo *et al.* 1985; Chiu *et al.* 1985; Gonda *et al.* 1985). In these phylogenetic analyses, heterogeneity of HIV strains has been neglected. We constructed a phylogenetic tree of different HIV strains, lentiviruses, and oncoviruses, by comparing amino acid sequences for the reverse transcriptase (RTase) and endonuclease/integrase (ENase) domains. This phylogenetic tree shows that among the HIV strains, BH5, BH10, pv. 22 isolated in New York and LAV_{BRU} in France, diverged most recently. Their common ancestor diverged from ARV2 in San Francisco somewhat earlier, and LAV_{MAL} and LAV_{ELI} in Africa are the most distantly related. It also confirmed that HIVs are related to the lentiviruses more closely than to oncoviruses. Using the rate of amino acid substitution for the *gag* protein, we also estimated the divergence times among the HIVs. For details, see *Jpn. Acad. Sci.* **63(B)**: 147–150.

Six Chloroplast Genes (*ndh*A-F) Homologous to Human Mitochondrial Genes Encoding Components of the Respiratory Chain NADH Dehydrogenase are Actively Expressed: Determination of the Splice Sites in *ndh*A and *ndh*B pre-mRNAs

Tohru MATSUBAYASHI, Tatsuya WAKASUGI, Kazuo SHINOZAKI
Kazuko YAMAGUCHI-SHINOZAKI, Norihiro ZAITA
Tadashi HIDAKA, Bing Yuan MENG, Chikara OHTO
Minoru TANAKA, Akira KATO, Takeo MARUYAMA
and Masahiro SUGIURA

Sequences (*ndh*A-F) homologous to human mitochondrial genes for components of the respiratory chain NADH dehydrogenase have been found in the chloroplast DNA of tobacco. The *ndh*A, D, E and F sequences corresponding to mitochondrial URF1, 4, 4L and 5 are located in a small single copy region, the *ndh*B sequence corresponding to URF2 in the inverted repeat and the *ndh*C sequence corresponding to URF3 in the large single copy region of the chloroplast DNA. Northern blot hybridization revealed that all six *ndh* sequences are actively expressed in the chloroplasts. The *ndh*A and *ndh*B sequences contain single introns and the splice sites of their pre-mRNA were determined by reverse transcription analysis. These findings suggest that potential components of an NADH dehydrogenase are synthesized in the chloroplasts. For details, see *Mol. Gen. Genet.* **210**: 385–393.

Male-driven Molecular Evolution Demonstrated by Different Rates of Silent Substitutions between Autosome- and Sex Chromosome-linked Genes

Takashi MIYATA*, Hidenori HAYASHIDA, Kei-ichi KUMA*
and Teruo YASUNAGA**

It is generally believed that the number of cell divisions differs between sperms and eggs (Winter, R. M. *et al.* (1983) *Hum. Genet.*, **64**, 156–159). This factor is important in relation to the rate of molecular evolution, because errors in DNA replication and repair are probably the major source of mutations contributing to molecular evolution (Wilson, A. C.

* Department of Biology, Faculty of science, Kyushu University.
** The Institute of Physical and Chemical Research.

et al. (1977) *Ann. Rev. Biochem.*, **46**, 573–639, Britten, R. J. (1986) *Science*, **231**, 1393–1398), as suggested by rapid evolutionary rates of viral-coded genes and mammalian mitochondrial genes (Hayashida, H. *et al.* (1985) *Mol. Biol. Evol.*, **16**, 23–36). We hypothesized the different numbers of germ-cell divisions between males and females result in different mutation frequencies between autosome and sex choromosomes, if replication errors are the major source of mutations.

Nucleotide sequence analyses for the highly divergent portions of autosome-, X- and Y-linked genes revealed a strong correlation between the observed rates of evolution and mutation frequencies expected from the model, when the male to female ratio of the number of germ-cell divisions is very large. These results suggest that males serve as a mutation generator in molecular evolution. For details, see *Proc. Japan Acad.*, **63**, 327–331.

Nucleotide Sequence of Immunoglobulin Epsilon Genes of Chimpanzee and Orangutan: DNA Molecular Clock and Hominoid Evolution

Yasuhiko SAKOYAMA*, Kyong-Ja HONG*, Si Myung BYUN*
Hiroshi HISAJIMA*, Shintaroh UEDA**, Yoshio YAOITA***
Hidenori HAYASHIDA, Takashi MIYATA****
and Tasuku HONJO***

To determine the phylogenetic relationships among hominoids and the dates of their divergence, the complete nucleotide sequences of the constant region of the immunoglobulin epsilon-chain (C_{e1}) genes from chimpanzee and orangutan were determined. These sequences were compared with the human epsilon-chain constant region sequence. A molecular clock (silent molecular clock), measured by the degree of sequence divergence at the synonymous (silent) positions of protein-encoding regions, was introduced for the present study. From the comparison of nucleotide sequences of alpha$_1$-antitrypsin and beta- and delta-globin genes between humans and Old World monkeys, the silent molecular clock was calibrated: the mean evolutionary rate of silent substitution was determined to be

* Department of Genetics, Osaka University School of Medicine.
** Department of Anthoropology, Faculty of Science, The University of Tokyo.
*** Department of Medical Chemistry, Kyoto University Faculty of Medicine.
**** Department of Biology, Faculty of Science, Kyushu University.

1.56×10^{-9} substitutions per site per year. Using the silent molecular clock, the mean divergence dates of chimpanzee and orangutan from the human lineage were estimated as 6.4 ± 2.6 million years and 17.3 ± 2.6 million years, respectively. It was also shown that the evolutionary rate of primate genes is considerably slower than those of other mammalian genes. For details, see *Proc. Natl. Acad. Sci. USA* **84**, 1080–1084.

Global Variation in G+C Content along Genome DNA Sequences with Reference to Chromosome Banding Structures of Vertebrate Genomes

Toshimichi IKEMURA and Shin-ichi AOTA

The global, rather than local, variation in the G+C content along the nuclear DNA sequences of various organisms was studied using GenBank Sequence Data. When genome portions of *E. coli* or of the yeast *S. cerevisiae* which had been sequenced for a long distance were examined, the levels of their G+C percentages were found to be within a narrow range around the overall genome G+C%. The G+C% levels of vertebrates, however, were found to lie over a wide range, showing that their genomes are mosaics of sequences with different G+C% levels, in which each of the sequences is fairly homogeneous in its G+C% for a very long distance. Through surveying the *Human Genetic Map* (HGM8, 1985; McKusic, 1986) and GenBank DNA sequences, we were able to connect the global variation in G+C% along the human genome at the sequence levels to the chromosomal G (Giemsa) and R (Reverse) band structures. For details, see *J. Mol. Biol* (in press).

Codon Usage Tabulated from the GenBank Genetic Sequence Data

Shin-ichi AOTA, Takashi GOJOBORI, Fumie ISHIBASHI
Takeo MARUYAMA and Toshimichi IKEMURA

Codon usages in 3681 genes were analyzed using all nucleotide sequence data obtained from the GenBank Genetic Sequence Data Bank (Release 50.0, 1987). This extensive survey of codon usage has recently been registered as a member of Molecular Biology Databases (LiMB Database by Los Alamos National Laboratory of USA) being designated CUTG (Codon

Usage Tabulation from the GenBank, by T. Ikemura).

To reveal the characteristics of the codon usage pattern for individual organisms, the frequency of codons in each organism was calculated by adding codon uses in all genes of the organism. The resultant codon-choice patterns are very similar among mammals, although the codon-choices (e.g. G+C% at the codon third letter) in individual genes of one mammal are often very different from each other. The codon-choice patterns, that are roughly common among mammals, do not depend on the choice of genes added; i.e., when the codon frequencies for ten or more genes with varying functions are summed up for each mammal, they usually converge into a very similar pattern regardless of the genes added. This converged pattern seems to relate both with the dinucleotide frequency of mammalian genomes and with their cellular tRNA content, although the pattern is clearly modulated by the segmental G+C% distribution along the genomes (*J. Mol. Biol.*, in press, 1988). See details, *Nucl. Acids Res.* **16**, Supplement, r315–r402.

Serological Survey of Mouse Complement Factor H in Common Laboratory and Wild-derived Strains; A New Allotype H.3

Yoshi-nobu HARADA, Kazuo MORIWAKI and Takeshi TOMITA*

Antigenic polymorphism of a serum protein migrating electrophoretically in the β region of mice has been reported as SAS-2 (serum antigenic substance-2) (Harada *et al. J. Immunogenet.* **14**: 33–41, 1987). By using serological analyses, SAS-2 was identified with complement factor H, which is a plasma glycoprotein of β-mobility functioning as a cofactor for the conversion of C3b to iC3b by serine protease I. Two allotypes of the factor H, H.1 and H.2, have been previously reported (Natsuume-Sakai *et al. J. Immunol.* **134**: 2600–2606, 1985). In the present study, a new allotype of complement factor H, H.3, was identified in BFM/2Ms and BFM strains derived from European wild mice. Using three different alloantisera for each mouse factor H allotype, a serological survey was carried out on common laboratory strains and wild-derived strains from *Mus musculus* and its relatives, *Mus spretus*, *M. spretoides*, and *M. spicilegus*. All the common laboratory strains examined in this survey had H.1 except

* Faculty of Agriculture, Nagoya University.

Table 1. Strain distribution patterns of factor H allotypes among common laboratory strains

Allotypes of factor H	Strains
H.1	A/J, AKR/J, BALB/c, BALB/cAnN, BALB/c-*Aph*-2^b CBA/J, CE/J, CFW, C3H/HeJ, C57BL/6J, C57BL/10J, C57BR, C57L, C58/J, DBA/1, DBA/2, DM, HRS/J, HTH, HTI, HTG, IITES, ITES, IXBL, KK, KR, KSB, LP/J, MA/MyJ, NC, NZB, P/J, PL/J, SM/J, SJL/J, SM/J, SWR/J, TF/GnLe, WB/ReJ, WHT, WN, 129/J
H.2	STR/N, BALB/c-*H*.2
H.3	not observed

for STR/N which had H.2 (Table 1). Geographical distributions of factor H allotypes in *M. musculus* were specific to the subspecies. Mice from *M. m. domesticus* and *M. m. castaneus* had the H.1 allotype. Mice from *M. m. musculus*, *M. m. bactrianus*, and *M. m. molossinus* had H.2. Only the BFM/2Ms and BFM strains derived from *M. m. domesticus* had H.3. Sera of strains derived from *M. spretoides* and *M. spicilegus* cross-reacted with anti-H.2 serum and those from *M. spretus* cross-reacted with anti-H.3 serum. A possibility was suggested that the gene for H.3 allotype in BFM/2Ms and BFM strains was derived from *M. spretus* by gene introgression.

Genetic Characterization of the Mouse Strain with Morphological Phenotypes Suggesting a Descendant from Ancient Oriental Stocks of Laboratory Mice (Japanese Waltzing Mice)

Hiromichi YONEKAWA, Jorn T. NIELSEN, Yoshibumi MATSUSHIMA
Shigeharu WAKANA, Hideki KATOH, Nobumoto MIYASHIMA
and Kazuo MORIWAKI

By the use of diagnostic genetical markers to distinguish subspecies of house mouse, *Mus musculus*, the origins of laboratory mice have been shown to be intersubspecific hybrids between the European subspecies, such as *M. m. domesticus* and an Asiatic subspecies such as "*M. m. molossinus*" or the Asiatic race of *M. m. musculus*". This conclusion is based on the following experimental evidence. Restriction analysis of mitochondrial DNA (mtDNA) showed that most of the laboratory strains

of mice have only one haplotype of mtDNA, suggesting that these strains including the strains called "old inbreds", descended from (a) small ancestral colony (ies) as (a) maternal founder(s). Restriction fragment length polymorphisms (RFLP) of nuclear genome using a DNA probe specific to the Y-chromosome, showed that the RFLP of most strains examined was identical to that of *M. m. musculus* but not of *M. m. domesticus*, suggesting that extensive introduction of paternal genes from the Asiatic subspecies had taken place during the establishment of recent laboratory mouse inbred strains. One remaining question about the origin of the Asiatic mice. whether the mice have a Japanese origin or a Chinese one.

Today there are many strains which show genetic characteristics very close to "*M. m. domesticus*", whereas no strain has been thought to exist which shows the characteristics being obviously descended from Asiatic mice. In the beginning of the 19th century, several investigators reported on laboratory stocks showing a distinctly Asiatic origin and they were commonly used for transplantation experiments for tumor cells. They were however thought to have become extinct during the development of research on laboratory mice, because there have been no reports on Asiatic mice since about 1940.

Two years ago, one of the authors, K.M., found a unique strain of laboratory mice, which had been bred by one of the authors, J.T.N., in the animal room of the University of Aahrus. The coat color and other morphological characteristics are very similar to those of ancient oriental stocks of laboratory mice described by Little and Tyzzer (1916) and Gate (1926) and they were called Japanese waltzing mice. If so, this strain would be important for following the history of laboratory mice. Thus we started to examine their genetic characteristics.

The restriction phenotypes of mtDNA show that the haplotype is identical to that of the Japanese wild mice "M. m. molossinus" but not those of any Chinese mice. A biochemical survey with mouse major tear protein complex (Mtp) and major salivary protein complex (Msp) also showed that both protein complexes of this strain were specific to Asiatic mice and not to any European mice, although this assay system could not distinguish between Japanese mice and other Asiatic mice. With a genetic survey of other biochemical markers specific to each chromosome, we found that this strain has *Hbbp* and *Pgm*-2b, which have hardly been found in laboratory mice. Other alleles examined were common among many other

strains of laboratorymice. These results strongly suggest that this mouse originated from Japanese mice but not from Chinese mice and that the strain is descended from the ancient stocks Asiatic mice such as the Japanese waltzing mice although this strain showed no waltzer phenotype.

Ribosomal DNA Spacer-length Polymorphisms in Rice

Yoshio SANO

Recent molecular studies have revealed that repetitive DNA families are widespread in genomes of higher organisms. One gene family that has been extensively studied in plants is the ribosomal gene family. The intergenic spacer regions of ribosomal RNA show extensive sequence divergence relative to the conserved coding regions. Both restriction-site variation and, more commonly, length variation occur, suggesting that the intergenic spacer regions in rDNA may evolve rapidly. Therefore, these intergenic spacer regions might be useful for tracing population level phenomena. We decided to study the evolution of this gene family in order to complement biosystematic studies of wild and cultivated rice. In rice, little is known about the extent of genetic variability and genetic behavior of rDNA repeats.

Spacer-length variation in rDNA was surveyed by Southern blotting analysis. Among 140 strains of wild and cultivated rice, 27 spacer-length phenotypes containing 13 different spacer-length variants have been detected so far. Length heterogeneity was observed in the spacer within and among species as well as within individuals. Asian cultivars and their wild progenitor were highly polymorphic for rDNA repeats whereas African cultivars and their wild progenitor were monomorphic. Ribosomal DNA repeats were differentiated among species as well as among varietal groups within Asian cultivars. However, all variants observed in cultivated species were found in wild progenitors, indicating that no variant is specific to cultivars. This implies that rDNA spacer-length variants have remained stable during the domestication process.

Rice Carries rDNA Repeats at Two Unlinked Loci

Yoshio SANO

Eukaryotic ribosomal DNA (rDNA) is organized into families of tam-

demly repeated genes, some or all of which may comprise the nucleolar organizer regions of chromosomes. Spacer-length heterogeneity within an individual was often observed in rice, especially in Asian wild rice. When heterogeneity was observed within the individual, these were two different size classes of rDNA repeats in most cases. In addition, the intensity of hybridization suggests that the classes are of roughly equal abundance. The question arose whether the two repeat lengths represent heterogeneity within the chromosome or are located in different chromosomes, each being homogenous for one class. In order to study the inheritance of rDNA spacer-length variants, F_2 segregations were observed in two selected crosses. Results indicated that at least 4 variants behave as Mendelian factors and rDNA repeats are located at two unlinked loci. Each variant specifes an array of tandemly repeated units as codominant allele. If rice carries rDNA repeats at two unlinked loci as shown here, it would appear that Asian cultivars tend to carry the same class of rDNA repeats at the two loci.

A Chromosomal Interchange Induced in a Near-isogenic Line Carrying an Alien Sterility Gene

Yoshio SANO

To understand the genetic mechanisms controlling sterility barriers between rice species, alien genetic factors which disturb the normal development of gametes were introduced into the genetic background of a standard strain by successive backcrossings, as previously reported (Ann. Rep. 33). A near-isogenic line of Taichung 65 was established from BC_8F_2. No other gene responsible for infertility was detected in BC_7F_2 and BC_8F_2. The extracted sterility gene (designated S_1) acted as a gamete eliminator in the heterozygous condition (S_1/S_1^a) and was tightly linked with *wx*. To purify the S_1 gene, backcrosses were continued. A different type of sterility factor was detected in BC_9F_1. The semi-sterile plants showed a normal segregation for waxy and nonwaxy pollen grains, suggesting that any gamete eliminator linked with *wx* no longer existed in the semi-sterile plants. The semi-sterile plants always segregated into a ratio of 1 fertile to 1 semi-sterile. A series of test crosses showed that the semi-sterility might be explained by the one-locus sporophytic interaction model. The model assumes that sporophytic semi-sterility occurs only in the heterozygote

(S/S^a) and no gametic selection occurs. Of paticular interest is the origin of the semi-sterility. It is apparent that a mutational event occured during the BC_8 to BC_9 transition.

The segregation patterns observed in the case of the sporo-gametophytic interaction model are also expected to occur in chromosomal interchanges although the two original cultivated species show no such chromosomal difference. If the semi-sterile plant showed no chromosomal abberation, an interchange of small segments might explain the infertility and, if so, the mutational event might involve a transposition of chromosomal segments. However, the semi-sterile plants showed a quardrivalent in the course of meiosis. This indicates that an interchange between non-homologous chromosomes occurred during the BC_8 to BC_9 transition.

IX. HUMAN GENETICS

Onocogene Activation by Philadelphia Translocations in Chronic Myeloid Leukemia and Acute Lymphoblastic Leukemia

Takashi IMAMURA and Hitoshi NAKASHIMA

The Philadelphia (Ph) chromosome is the result of a reciprocal translocation between the long arms of chromosomes 9 and 22 t(9; 22)(q34; q11). The breakpoints on chromosome 22 band q11 occur in a small region of 5.8 kb, the breakpoint cluster region or *bcr*. Subsequent cloning experiments and DNA sequence analysis revealed that the *bcr* is part of a large gene, which has been called the *bcr* gene. The *bcr* contains four exons (b1–b4), and chromosomal breaks occur in two introns between exons b2, b3, and b4. Breakpoints on chromosome 9 are scattered over a distance of at least 100 kb, but are all located 5′ of the tyrosine kinase domain of the *c-abl* proto-oncogene. As a result of the Ph translocation, the *c-abl* gene is transferred from its normal position on chromosome 9 band q34 to the Ph chromosome. This event creates a head to tail *bcr-abl* juxtaposition on the Ph chromosome, with the bcr gene closer to the centromere.

The presence of the Ph chromosome was thought to be pathognomonic for CML. However, patients with acute lymphoblastic leukemia (ALL) have a high percentage of Ph+ marrow cells. To date, it has been reported that 25%–30% of adult and 2%–10% of childhood ALL are Ph positive. It is surprising that Ph+ ALL and CML share the same marker chromosome, while they are clinically different diseases. Either the two diseases are different expressions of one underlying malignant process, or the marker chromosome is not the same at the molecular level. A factor obscuring the difference between Ph+ ALL and CML is the fact that following the chronic phase, 30% of the CML patients eventually develop a lymphoid blast crisis that closely resembles *de novo* Ph+ ALL.

We report here an studies on the underlying genetic mechanism in Ph+ bcr− ALL. Chromosomal breaks still occur within the bcr gene, but they take place in the putative first intron of the bcr gene, which is 5′ of the *bcr* involved in CML. In this way an alternative *bcr-c-abl* fusion gene is

created, which is transcribed into a chimeric 7 kb *bcr-c-abl* mRNA. Most likely the RNA encodes a Ph+ ALL-specific p190 *bcr-abl* hybrid protein since this product was shown to be present in another Ph+*bcr*− ALL patient with a chromosomal breakpoint in the first *bcr* intron. These findings establish the existence of two molecularly distinct Ph chromosomes in Ph+ ALL versus CML.

Malignancy is probably a multistage process involving a series of genetic changes rather than a single mutational event. One such genetic change in the pathogenesis of CML appears to be the acquisition of the Ph. The enhanced expression of *abl*-related RNA in the K562 cell line is associated with amplification and rearrangement of the *c-abl* gene in these cells. To determine if the similarly enhanced expression of *c-abl* in the blast crisis cells was also associated with *c-abl* gene amplification or rearrangement, the *v-abl* probe was hybridized to Southern blots of DNA from 2 blast crisis cells, digested with restriction endonucleases. In contrast to K562, intense hybridization of the *v-abl* probe to DNA from these two cells was not noted, indicating that *c-abl* sequences were not amplified in these cells. Thus, the increased *c-abl*-related RNA in these CML blast crisis cell lines may arise through different pathways: in some cells, including K562, *c-abl* gene amplification may be the major pathway, whereas in others the pathway is unknown.

We and others have showed that in Ph+*bcr*− ALL, breakpoints in chromosome 22 occur within the same *bcr* gene, but at 5′ more of the *bcr*. This resulted in deletion of most of the exons of the *bcr* gene from the *c-abl*-related mRNA because of translocation of the *bcr* exon regions to chromosome 9. Thus the deletion of the rearranged *bcr*/*c-abl* sequences at the 5′ side on the CML blast crisis may result in the manifestation of a much more aggressive leukemic state which differs from those usually observed in chronic phase of CML.

In a series of DNA transfection analyses using the tumorigenicity assay, we observed no tumor induction in nude mice in four of the CMLs investigated, which is in contrast to the previous report in that four of seven AMLs and all three AML cell lines tested actually induced tumor formations. The analysis for the presence of mutated *RAS* genes by means of hybridization of mutation-specific oligonucleotide probes to genomic DNA has been considerably improved by including an *in vitro* amplification step of *RAS* sequences. Investigation of 6 CML in chronic acute or phase and

the K562 cells, all positive for Ph chromosome, gave no indication for mutated *RAS* genes. These results are comparable with DNA transfection studies that likewise detected activated *RAS* sequences in CML very infrequently. These results indicate that in Ph positive CML and ALL there appers to be no case which contains activated *RAS* sequences.

Evolutionary Implications of Mitochondrial DNA Polymorphism in Human Populations: Comparison of Two Different Populations in Japan

Satoshi HORAI, Kenji HAYASAKA, Takashi GOJOBORI and Ei MATSUNAGA

In a previous study (Horai and Matsunaga 1986), we analyzed 116 samples of mtDNAs from Japanese living in Shizuoka Prefecture located in central Japan. A total of 95 different morphs were detected with 9 enzymes (HaeIII, HinfI, Sau3AI, HhaI, RsaI, TaqI, HpaII, AvaII and AccII), 60 of which had not been documented previously. Based on a comparison of the cleavage maps of all individuals, 62 types of different combinations of the morphs were observed. A phylogenetic analysis of the 62 types indicated that they are separated into at least two distinct groups with different frequencies: a group with the smaller frequency (group I; 18%) which first diverged from the other group with the larger frequency (group II; 82%). The divergence time between the two groups was estimated to be as early as 125,000 years ago, assuming a rate of mtDNA divergence as 2×10^{-8} per site per year. However, this dating seems too early, if we accept that racial divergence took place about 120,000 years ago, as estimated from gene frequency data for blood proteins (Nei and Roychoudhury 1974). Therefore we intended to investigate whether or not similar clustering patterns could be observed in Okinawa, which is located about 1,300 km from Shizuoka.

A total of 82 mtDNAs from the Okinawa people (Ryukyuan) were analyzed with the same nine enzymes as used previously. Sixty different morphs were observed in total, 19 of which were unique to that population. Comparisons of the frequencies of each morph for the Okinawa population with those for the mainland Japanese revealed significant differences in the frequencies of 10 morphs between the two populations. In particular,

HinfI morph 31 was observed at a frequency of 35% in Okinawa, while the frequency of this morph was only 11% on the mainland. Moreover, in the RsaI digestions, 17 different morphs were observed on the mainland, whereas only 6 morphs were detected in Okinawa. Furthermore, HaeIII morph 2 was found at the frequency of 5% in Okinawa, while this morph was observed at the frequency of 16% on the mainland. These observations therefore indicate that a considerable genetic difference exists between the Okinawa and mainland populations. However, all individuals who possessed HaeIII morph 2 in the Okinawa population shared five additional variant morphs with those for the mainland. These five morphs are HaeIII morph 35, HinfI morphs 27 and 28, RsaI morph 13 or 17, and TaqI morph 15. As will be discussed later, these individuals belong to group I in the phylogenetic tree constructed for mainland Japanese.

Based on a comparison of the cleavage maps among the Okinawa samples, 39 types of different combinations of the morphs were observed. It is remarkable that only 11 of them have been found among mainland Japanese while the remaining 28 types were unique to the Okinawa population. The combination of two variant morphs (HaeIII morph 3 and HinfI morph 31) were scored in 17 individuals, though none of the mainland Japanese showed this type. We then estimated the numbers of nucleotide substitutions (d) for each pair of these types using the method of Nei and Li (1979). The mean value for d was 0.0016, taking into account the frequency of each type, which is lower than that (0.0026) for mainland Japanese.

A phylogenetic tree among the 39 types within the Ryukyuans was also constructed by the unweighted pair group (UPG) method (Sokal and Sneath 1963; Nei 1975). The overall pattern of the tree was very similar to that for the mainland Japanese. The Okinawa population can also be separated into two distinct groups which correspond exactly to groups I and II from the mainland. Moreover, the divergence time between the two groups in the Okinawa tree was estimated to be about 125,000 years ago (Genetic distance $D=0.005$), which was virtually the same as that obtained from the mainland Japanese. However, group I included 4 types (4 individuals) while the remaining 35 types (78 individuals) belonged to group II. The divergence time of the earliest branch point for group I of the Okinawa population was estimated to be about 95,000 years ago ($D=0.004$), whereas all types in group II seem to have diverged only 75,000 years ago ($D=0.003$). The recent divergence of all types within group II apparently

contributes to the low mean value of d in the Okinawa population.

Phylogenetic Relationships among Japanese, Rhesus, Formosan, and Crab-eating Monkeys Inferred from Restriction Enzyme Analysis of Mitochondrial DNAs

Kenji HAYASAKA, Satoshi HORAI, Takashi GOJOBORI
Takayoshi SHOTAKE, Ken NOZAWA and Ei MATSUNAGA

About 20 species of macaques, genus *Macaca*, are widely distributed throughout Asia as well as neighboring islands having adapted to diverse environments. While they can be distinguished from each other by their morphological traits, some of them inhabit sympatrically and interbreed. This implies that they have not speciated completely and are in the process of speciation. Therefore, genetic studies of macaques are of interest for understanding the processes of evolution.

We, thus, analyzed mitochondrial DNA (mtDNA) polymorphisms in four species of macaques, i.e., ten Japanese monkeys, *Macaca fuscata*, one rhesus monkey, *M. mulatta*, one Formosan monkey, *M. cyclopis*, and one crab-eating monkey, *M. fascicularis* to study phylogenetic relationships.

Using 17 restriction enzymes of six base pair recognition, 42 to 49 sites were observed in the samples. The estimated number of nucleotide substitutions per site among Japanese, rhesus, and Formosan monkeys ranged from 0.0318 to 0.0396 and that between crab-eating and the other monkeys from 0.0577 to 0.0653. The phylogenetic trees constructed from these values suggested the following relationships; The four types of Japanese monkeys form a culster and the crab-eating monkey appears the most distantly related to the others. The Formosan monkey diverged from the lineage leading to Japanese and rhesus monkeys before the latter two species diverged. However, an exact branching order for these three species cannot be conclusively determined by this analysis, because of the small differences in estimated nucleotide diversity among these species and the considerable sampling error due to the small number of recognition sites. If we assume an average nucleotide substitution rate of $(2\text{–}4)\times 10^{-8}$ per site per year (Wilson *et al.* 1985), the Japanese, rhesus, and Formosan monkeys would have diverged approximarely 0.9–1.8 million years (Myr) ago and the divergence time between these three monkeys and the crab-eating monkey would be 1.5–3.0 Myr ago.

The phylogeny suggested from our analysis is compatible with that suggested from fossil records in terms of topology but not for divergence times. This discrepancy might result from the ambiguity of the estimation of divergence times from fossils. Alternatively, it is also possible that we overestimated the divergence times since what we estimated was the divergence time for genes but not for species.

The analysis of blood protein polymorphism suggested that phylogenetic relationships differ from those we found. This discrepancy might have been caused by a difference in mechanisms of inheritance between nuclear DNA and mtDNA since mtDNA is inherited maternally unlike nuclear DNA.

Female macaques stay in their native populations for their entire lives, while males migrate between populations. In the past, there might have been a large extent of overlapping distribution. This is partly because the Asian continent had been contiguous to its neighboring islands from time to time. Moreover, hybrids between rhesus and crab-eating monkeys have been observed in nature. Interspecific hybrids of macaques have also been observed in captivity and are known to be fertile. It is possible that bisexually inherited nuclear genomes might have been homogenized by the migration of males while maternally inherited mtDNA might reflect the past events of population splitting more accurately. For details, see *Mol. Biol. Evol.* **5**(3), 270–281.

X. APPLIED GENETICS

Alien Pollen Primacy (APP) in the Rice Plant

Yo-Ichiro SATO, Ling-Hua TANG* and Ryuji ISHIKAWA**

Mendel's law assumes that all pollen grains deposited on stigma have the same ability in terms of fertilization. Segregation ratios at a marker locus will be distorted from the expected ratio 3: 1, when pollen grains having a certain allele at the locus show either a higher or lower ability for fertilization than ones having different alleles. Until recently there have been three recognized cases when Mendel's assumption would be at fault; 1) differential germination rates or elongation speeds of pollen tubes are brought about by particular certation genes or various environments, Alien Pollen Primacy (APP) was recently discovered as the fourth case in which Mendel's law could not be adopted.

APP was found in a mixed pollination experiment in rice conducted in the summer of 1987. Four kinds crosses using two varieties, A and B, *i.e.*, $A\times(A+B)$, $A\times(B+A)$, $B\times(A+B)$ and $B\times(B+A)$ were made and all offspring were grown in the winter season of 1987–1988. $A+B$ denotes A pollen being deposited five minutes earlier than B pollen on the same stigma. The amount of A pollen and that of B pollen were approximately the same. In each cross, progeny fertilized by A pollen or B pollen were estimated using particular gene markers. In the first cross, the number of offspring fertilized by A and B pollen was approximately equal, even though A pollen was deposited prior to B. In the second cross, offspring fertilized by B were three times more frequent than those of A. These two crosses indicated B pollen showed an advantage over A pollen for fertilization. The latter two crosses in which B was used as the female parent, however, showed a strong advantage for A pollen. The data obviously indicates that between A and B, plants tended to accept alien pollen more frequently than their own pollen. This feature of APP seems to be common to self-incompatibility by means of an advantage for alien pollen. In case of self-incompatibility, own pollen cannot be accepted, and self-pollination results in failure. In common rice, all varieties are self-com-

* Jiangsu Academy of Agric. Science, Nanjing, China.
** Hokkaido Univ., Present address: Hirosaki Univ.

patible, and therefore, APP should be distingushed from self-incompatibility.

The *Ph-Aph* Association in Rice, and its Significance in Indica-Japonica Differentiation

Yo-Ichiro SATO and Hiroko MORISHIMA

A complicated association between genes and characteristics was observed among native cultivars as well as in hybrid populations raised by the single-seed-descent method (SSD) from an Indica × Japonica cross in common rice. SSD is a breeding method that minimizes zygotic selection. Many associations found among native cultivars disappeared in the F_2 generation, indicating that they were shaped through selection explored by cultivation under various environments. Association observed in F_2 were due to pleiotropy or linkage of the genes concerned. Several associations which were observed in native cultivars but not in F_2, *e.g.*, that between positive phenol reaction (*Ph*) and short apiculus hair length (*aph*) (*Ph-aph* association), were detected in F_5 raised by SSD. *Ph* and *Aph* loci are located on different chromosomes. Thus the *Ph-aph* association can not be caused by zygotic selection nor linkage.

It was suggested that a particular gametophyic selection or certation brought about this association.

Intra-Populational Genetic Diversity Found in Indigenous Rice Cultivars of Yunnan

Hiroko MORISHIMA

Two population samples of primitive rice cultivars were obtained from a farmer's seed stock in Yunnan, China. One is an upland population and one a lowland population. Variation and covariation within populations were examined regarding several agronomic characteristics supposedly related to adaptability to water conditions, as well as diagnostic characteristics and isozymes which are useful for the classification of Indica-Japonica types representing the two major varietal groups of Asian common rice.

The lowland population was relatively uniform, consisting of Indica plants

with lowland adaptability. In contrast, the upland population showed a large amount of variability ranging from Indica to Japonica types, and contained, in addition to upland-type plants, some lowland-type plants. The average gene diversities computed from 13 polymorphic loci were 0.099 and 0.153 for lowland and upland populations, respectively.

In each population, grain yield was evaluated under two different diversity levels; first, as an average of component lines which were each derived from original plants and tested in pure stands, and secondly, as a population composed of a mixture of one seed from one component line. The upland population, though it was a lower yielder than the lowland one under our experimental conditions, tended to show an "populational buffering" or an advantageous effect due to genotype mixture.

Marked difference in diversity level found between two natural populations cultivated by the same farmer suggests a differential effect imposed by lowland and upland conditions on preserving genetic diversity.

Characteristics of Weed Rice Strains Collected from Various Asian Countries

Ling-Hua, TANG and Hiroko MORISHIMA

A weed is defined as an unwanted plant which has adapted to habitats disturbed by man. Many crops are accompanied by their related "weed" types, in addition to their "wild" progenitors. The weed type of a certain crop might be 1) a transitional state from wild to cultivated type, or 2) an escape from cultivars, or 3) derivatives of natural hybridization between wild and cultivated types. In rice, the existence of weed types in and around arable lands (mainly rice fields) was reported in many localities of rice growing areas. Because weed rice can be said, in a sense, to be an intermediate state between wild and cultivated types, studies on its origin and genetic nature might provide important information for understanding the evolution of rice and also for practical rice breeding.

Twenty-four weed rice strains obtained from many Asian countries were examined regarding various characteristics and isozyme loci. Each strain was quantitatively evaluated on the axes of wild vs. cultivated variation and Indica vs. Japonica variation which represents a major varietal differentiation in Asian common rice.

1) Weed rice strains possessed intermediate characteristics between wild

and cultivated types showing a wide variation. Some were similar to wild types in that they have high degree of seed shedding, seed dormancy, and black and light seeds, though they looked like cultivars at the stage of vegetative growth. Others were very similar to cultivated types in many respects, being distinguished only by a red pericarp. Such "mimics" of cultivars are probably harvested and sown together with cultivated rice unconsciously.

2) Judging from several diagnostic characteristics and allozymes which are useful for Indica-Japonica classification, weed strains collected from China (Jiangsu) and Korea where Japonica types are cultivated were of the Japonica type, while those from India and Thailand where Indica types are cultivated were close to the Indica type, though variation was large. Interestingly, those collected from hilly areas of Nepal, where both Japonica and Indica types were grown within the same field, were found to be Indica-Japonica intermediates.

Weed rices found in humid tropics inhabited by wild rices originated most probably from the natural hybridization between wild and cultivated types followed by selection for weediness. However, the origin of weed rices found in areas where wild rice is not distributed remains unknown.

Isozyme Genes Useful for Indica-Japonica Classification of Common Rice

Ling-Hua TANG and Yo-Ichro SATO

Two subspecies of common rice, Indica and Japonica types, differ not only in so-called discriminating characteristics but also in allelic frequencies at various isozyme loci (Glaszmann 1987; de Kochiko 1987). It is expected that an adequate combination of alleles at a fewer number of enzyme-coding loci can effectively discriminate between Indica and Japonica types. To look for such combinations alleles at twelve isozyme loci, *Amp-1*, *Amp-2*, *Amp-3*, *Acp-1*, *Cat-1*, *Est-2*, *Est-9*, *Pgd-1*, *Pgi-1*, *Pgi-2*, *Pox-2* and *Sdh-1*, of 104 cultivars from various localities in Asia were examined. The cultivars were classified into Indica and Japonica types prior to isozyme analysis, using Sato's discriminant function.

$$Z=Ph+1.313K-0.82Hr$$

where Ph, K and Hr indicate the phenotypic value of phenol reaction,

susceptibility to $KClO_3$ and apiculus hair length respectively, (Sato *et al.* 1986). The hundred and four cultivars tested were classified into 51 Indicas and 53 Japonicas.

It was pointed out that between the Indica and Japonica types allelic frequencies significantly differed at 10 loci other than *Amp-1* and *Sdh-1*. Particularly at *Amp-2*, *Acp-1* and *Cat-1* loci allelic frequencies were markedly different between the two types. Allele combinations at these three loci may be used for making a rough estimate whether a cultivar falls into the Indica or the Japonica type. Of 51 classified as Indica type from their Z value, 47 commonly had $Amp\text{-}2^2$, $Acp\text{-}1^{-4}$ and $Cat\text{-}1^1$ (designated as (2, −4, 1)). Fourty-seven out of 52 classified as Japonicas had an association (1, 9, 2) which was the diametric combination of (2, −4, 1). To confirm this trend, 48 random samples from upland native cultivars in Thailand were used and subjected to the same analysis. Forty-four had (2, −4, 1) or (1, 9, 2), and the former largely belonged to the Indica and the latter to the Japonica type. Probability of misjudgement was less than 10 percent.

Location of *Pgd-1* Locus in Rice

Ryuji ISHIKAWA*, Toshiro KINOSHITA* and Hiroko MORISHIMA

One of the rice isozyme loci, *Pgd-1*, was found to be located on chromosome 9 (linkage group VIII) by trisomic analysis (Ishikawa 1986). To determine the location of *Pgd-1* on this chromosome by linkage analysis, an Indica strain carrying allele 2 at the *Pgd-1* locus was crossed with a Japonica tester strain F1-280 which has an alternative allele 2 at this locus and two mutant genes, *la* (lazy) and *v*-4 (virescent) belonging to linkage group VIII. F_2 segregation indicated *Pgd-1* was linked with these two loci. The recombination value was calculated using the formula of Allard (1956). *Pgd-1* showed a recombination of 5.4% with *v*-4, and 14.2% with *la*. In addition, a linkage between *Pgd-1* and another isozyme locus *Adh-1* was found, though the order of their sites has not yet been determined. Thus, twelve isozyme loci were so far assigned in rice to their relevant chromosomes and four of them were mapped.

* Hokkaido University.

Genetic Aspects of Differentiation into Annuals and Perennials in Asian Wild Rice

Pascale BARBIER* and Hiroko MORISHIMA

Surveys of inter-populational variation in Asian common wild rice *Oryza rufipogon* (Asian form of *O. perennis*) showed that various characteristics pertaining to the life-history and mating system are intercorrelated in the following way: Early flowering, high fecundity, low post-reproductive survival and selfing behavior on one hand (characterizing the annual type), and late flowering, low fecundity, high post-reproductive survival and outbreeding behavior on the other hand (perennial type).

To investigate the genetic basis of these characteristic associations, four crosses were made between individuals sampled from one annual population (used as females) and those from one perennial population (males), and the pattern of covariation between characteristics in F_2 was examined. Many of the correlations mentioned above were not found in the F_2 progeny of these crosses. This indicates that such correlations may have no genetic basis such as by linkage or pleiotropy. The corresponding trait associations which were observed in the interpopulation surveys probably resulted from independent selection of these traits. This is consistent with the rather low intra-population variance observed both in annuals and perennials. However, a positive correlation between early flowering and panicle number (the main component of fecundity) was consistently found in F_2s of all crosses ($r=0.377-0.523$, $n=50-60$). Other significant correlations were found in some crosses; for example the fecundity component (panicle number) and the longevity component (number of vegetative tillers developing after reproduction) were negatively correlated ($r=0.314$), longevity and outcrossing rate (estimated from anther length) were positively correlated ($r=0.390$).

An investigation of relationships between these life-history traits and allozyme variation revealed an association between the presence of alternative alleles at locus *Pox-1* and early *vs.* late flowering. Interestingly, this association is also found among natural populations; the mechanism whereby such association persists in nature has however not been elucidated.

* Nagoya University.

Electrophoretic Analysis by Immobilized pH Gradient of Seven Protein Fractions Sequentially Extracted from Rice Endosperm

Toru ENDO

From 10 g of 100-mesh rice endosperm powder, seven fractions of four protein classes were sequentially extracted. In order of extraction, they are as follows: acid-soluble (alb-1) and alkali-soluble albumin (alb-2), acid-soluble (glb-1) and alkali-soluble globulin (glb-2), alcohol-soluble prolamin (prl), and acid-soluble (glt-1) and alkali-soluble glutelin (glt-2). Each extract was lyophilized immediately, or following desalting, or was dried after centrifugation. The protein contents of the powdered extracts were estimated by the CBB method. The extracts were then dissolved in 8M urea and subjected to electrophoresis in an acrylamide gel (4% T, 3% C) with an immobilized pH 4–10 gradient. A sample of the results are shown in Fig. 1.

From the figure, the numbers of bands in each of the seven fractions were

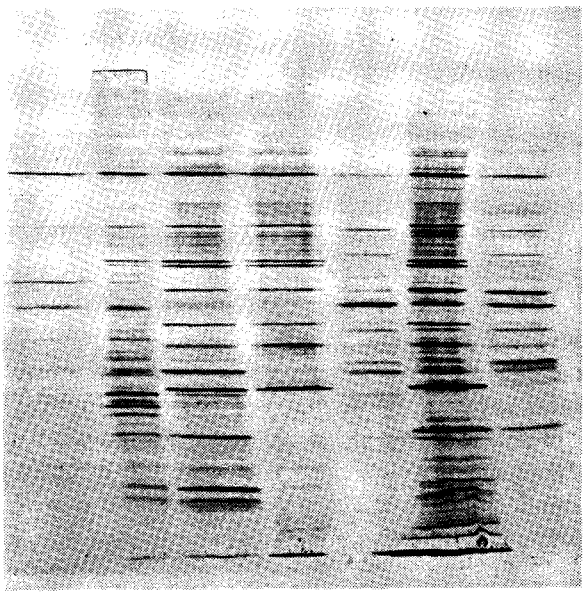


Fig. 1. Anode at top. From left, samples applied are alb-1, alb-2, glb-1, glb-2, prl, glt-1 and glt-2 of rice endosperm of cv. Inakura, tropical type of Japonica.

counted. For the alb-1 through glt-2 fractions the numbers were: a minimum of 42 (including 3 major bands), 59(11), 66(14), 52(8), 32(6), 60(12), and 33(7), respectively. It can be seen that there are appreciable differences between the acid-soluble and alkali-soluble fractions of the three protein classes, i.e. albumin, globulin, and glutelin. Also, there are similarities between the band patterns of the alb-2 and glb-2 fractions as well as those of the glb-2 and glt-1 fractions. These observations suggest that the extractants used may be inadequate relative to the complexity of the endosperm protein molecules.

Multiple Alleles at the wx Locus as a Major Factor for Amylose Content in Rice Grain

Yoshio SANO

Rice cultivars greatly differ in amylose content, the major determinant of grain quality. As reported already (Ann. Rep. 34), there are two alleles, Wx^a and Wx^b, in addition to *wx* which control the quantitative level of amylose as well as the gene product called *Wx* protein. Therefore, the two alleles of Wx^a and Wx^b were considered to be a major factor controlling amylose content among nonglutinous cultivars. However, considerable variations in both amylose content and *Wx* protein levels are found among cultivars, with some cultivars showing an intermediate amount between those of Wx^a and Wx^b. One possible explanation for this is that there may be other genes which regulate both amylose content and *Wx* protein level such as low amylose mutant genes. Another possible explanation is that there are additional *Wx* alleles which produce an intermediate amount of amylose and *Wx* protein between those of Wx^a and Wx^b. To investigate this, *Wx* genes were introduced into a standard glutinous strain (Taichung 65 *Wx*) by successive backcrosses from three cultivars which produced an intermediate amount of amylose and *Wx* protein. In BC_3F_2 and BC_4F_2, homozygous plants for *Wx* always showed an intermediate amount of amylose and *Wx* protein. Wx^a and Wx^b were not detected in the derivatives. This implies that an additional *Wx* allele also contributes to variations in amylose content among nonglutinous cultivars.

XI. DATABASE

Constructing a DNA Database

Sanzo MIYAZAWA

A data entry system and data search/retrieval system for DNA sequence data were developed on a unix operating system. We built a data entry system by utilizing the SCCS (Source Code Control System) available in the unix operating system. The SCCS is a source code management system with such features as version control and exclusion control. Version control means that a record is kept with each set of changes; of what the changes are, why they were made, and who made them and when. Ecclusion control means that only one person can modify data at a time. Both are critical in data entry with more than one user. Quality control of data is also important in any database. We installed programs made by Dr. J. Fickett in the GenBank for error checking of data. These programs check whether the following features are illegal or not; record format, journal name, start and stop codons, and codon frame. A codon table which depends on an organism is maintained as a database. Taxonomy records in the data are automatically generated by using a taxonomy database which is also maintained by GenBank. More extensive automatic error-checking should be developed on the basis of studies of the telationship between DNA sequence pattern and function. A simple search and retrieval system which uses flat files and therefore called "flat" has also been developed. Some basic tools are available as filters in the unix system to output specified types of records, to search strings expressed in regular expression over database and output names of entries which include such strings, to take "and", "or" and "xor" over sets of entry names, and to cut out specified entries from the database. By joining such filters together with "pipe", one can search and retrieve entries from the database by keywords such as author name, journal name, title, organism name, gene name, and any combination of such items. This "flat" system is designed to be portable and easy to maintain at the cost of speed; it is portable among unix systems which are available for a wide range of computers from super to personal computer.

Publication from Genetic Resources Section, Genetic Stocks Research Center

Shin-ya IYAMA

The following publications were released from the Genetic Resources Section this year.

1. "Experimental Mouse Strains Maintained in Universities in Japan. 1987." (Compiled by S. Iyama and K. Moriwaki) 177 pages (in Japanese). This was completed in cooperation with the Mammalian Section of this Center. The catalogue listed 1032 entries of 621 genetic strains of mouse maintained in various universities and research institutes in Japan under the administration of the Ministry of Education and Culture. Each entry was described with its strain name, location maintained, historical record of acquisition, genetic characteristics, and information on maintenance conditions and distribution.

2. "Rice Genetics Newsletter Volume 4". 131 pages (in English). This is published in cooperation with the Rice Genetics Cooperative, an international organization of rice geneticsts. This volume contained the following:

(1) Eleven newly registered gene symbols approved by the Committee on Gene Symbolization, Nomenclature and Linkage Groups.

(2) Lists of newly adopted gene symbols (20) and marker genes (25) whose linkage groups have been confirmed. These are in supplement to lists appearing in previous issues.

(3) Current linkage maps of rice.

(4) A List of 60 recently published papers of rice genetics.

(5) 32 research notes of various fields of rice genetics.

PUBLICATIONS

AOKI, K.: Gene-culture waves of advance. J. Math. Biol. **25**: 453–464, 1987.

AOKI, K.: Adult lactose absorption and milk use from the standpoint of gene-culture theory. Jpn. J. Genet. **62**: 445–459, 1987.

AOKI, K. and FELDMAN, M. W.: Toward a theory for the evolution of cultural communication: coevolution of signal transmission and reception. Proc. Natl. Acad. Sci. USA **84**: 7164–7168, 1987.

AOTA, S., GOJOBORI, T., SHIGESADA, K., OZEKI, H. and IKEMURA, T.: Nucleotide sequence and molecular evolution of mouse retrovirus-like IAP elements. Gene **56**: 1–12, 1987.

BONHOMME, F., GUENET, J.-L., DOD, B., MORIWAKI, K. and BULFIELD, G.: The polyphyletic origin of laboratory inbred mice and their rate of evolution. Biol. J. of the Linnean Soc. **30**: 51–58, 1987.

BANDO, H., KUSUDA, J., GOJOBORI, T., MARUYAMA, T. and KAWASE, S.: Organization and nucleotide sequence of a densovirus imply a host-dependent evolution of parvoviruses. J. Virology **61**: 553–560, 1987.

BOURSOT, P., YONEKAWA, H. and BONHOMME, F.: Heteroplasmy in mice with deletion of a large coding region of mitochondrial DNA. Mol. Biol. Evol. **4**: 46–55, 1987.

ENDO, T.: Differential characteristics of endosperm protein fractions between Indica and Japonica rice varieties. In "*Crop Exploration and Utilization of Genetic Resources*" (S.C. Hsieh, ed.), pp. 31–39, Taiwan Prov. Taichung Dist. Agric. Imp. Stat., 1987.

FISCHER-LINDAHL, K., LOVELAND, B., RICHARDS, S. and YONEKAWA, H.: In search of the molecular basis for a mitochondrial transplantation antigen. Abstract of papers presented at the 1987 meeting on molecular biology of mitochondria and chloroplasts. p. 205. Cold Spring Harbor Laboratory, 1987.

FUJII, T., HUANG, Y.-D., HIGASHITANI, A., NISHIMURA, Y., IYAMA, S., HIROTA, Y., YONEYAMA, T. and DIXON, R. A.: Effect of inoculation with *Klebsiella oxytoca* on dinitrogen fixation by rice-bacteria association. Plant and Soil **103**: 221–226, 1987.

FUJII, T. and INOUE, T.: Modulating effect of dimethylbenzanthracene on gamma-ray mutagenesis in the soybean test system. Jpn. J. Genet. **62**: 425–430, 1987.

FUJISAWA, T.: Inhibition of stenotele differentiation by head tissue in hydra. J. Cell Sci. **87**: 315–322, 1987.

FUJISAWA, T.: An endogenous inhibitor involved in position-dependent stenotele differentiation in hydra. Dev. Biol. **122**: 210–216, 1987.

FUJITA, N., NOMURA, T. and ISHIHAMA, A.: Promoter selectivity of *Escherichia coli* RNA polymerase: Purification and properties of holoenzyme containing the heat-shock sigma subunit. J. Biol. Chem. **262**: 1855–1859, 1987.

FUJITA, N., ISHIHAMA, A., NAGASAWA, Y. and UEDA, S.: RNA polymerase sigma-

related proteins in *Escherichia coli*: Detection by antibodies against a synthetic peptide. Mol. Gen. Genet. **210**: 5–9, 1987.

FUJITA, N. and ISHIHAMA, A.: Heat-shock induction of RNA polymerase sigma-32 synthesis in *Escherichia coli*: Transcriptional control and a multiple promoter system. Mol. Gen. Genet. **210**: 10–15, 1987.

GLASS, R. E., JONES, S. T. and ISHIHAMA, A.: RNA polymerase is a target for ppGpp. In "*RNA Polymerase and the Regulation of Transcription*", (W. S. Reznikoff, *et al.*, eds.), pp. 393–396, Elsevier Science Publ., New York, 1987.

GLASS, R. E., JONES, S. T., NOMURA, T. and ISHIHAMA, A.: Hierarchy of the strength of *Escherichia coli* stringent control signals. Mol. Gen. Genet. **210**: 1–4, 1987.

GOJOBORI, T., MORIYAMA, E. N. and YOKOYAMA, S.: Molecular evolution of oncogenes. Jpn. J. Genet. **62**: 163–177, 1987.

GOJOBORI, T. and YOKOYAMA, S.: Molecular evolutionary rates of oncogenes. J. Mol. Evol. **26**: 148–156, 1987.

HARADA, Y.-N., HAYAKAWA, J.-I., NODA, E. and TOMITA, T.: A gene locus controlling a serum protein migrating electrophoretically in the β region of mice and detected by using a strain derived from the Japanese wild mouse (*Mus musculus molossinus*). J. Immunogenet. **14**: 33–41, 1987.

HAYASHI, J.-I., YONEKAWA, H., MURAKAMI, J., TAGASHIRA, Y., PEREIRA-SMITH, O. M. M. and SHAY, J.: Mitochondrial genomes in intraspecies mammalian cell hybrids display codominant or dominant/recessive behavior. Exp. Cell Res. **172**: 218–227, 1987.

HIAI, H., BUMA, Y. O., IKEDA, H., MORIWAKI, K. and HISHIZUKA, Y.: Epigenetic control of endogeneous ecotropic virus expression in SL/Ni mice. JNCI **79**: 781–787, 1987.

HIROSE, S. and SUZUKI, Y.: *In vitro* transcription of eukaryotic genes are affected differently by the effect of DNA supercoiling. Proc. Natl. Acad. Sci. USA **85**: 718–722, 1988.

HIROSE, S., TABUCHI, H. and YOSHINAGA, K.: GTP induces knotting, catenation and relaxation of DNA by stoichiometric amounts of DNA topoisomerase II from *Bombyx mori* and HeLa cells. J. Biol. Chem. **263**: 3805–3810, 1988.

HONDA, A., UEDA, K., NAGATA, K. and ISHIHAMA, A.: Identification of the RNA polymerase-binding site on genome RNA of influenza virus. J. Biochem. **102**: 1241–1249, 1987.

HORAI, S., GOJOBORI, T. and MATSUNAGA, E.: Evolutionary implications of mitochondrial DNA polymorphism in human populations. In "*Human Genetics*" (F. Vogel and K. Sperling, eds.), pp. 177–181, Springer-Verlag, Berlin, 1987.

HORAI, S., INOUE, T. and MATSUNAGA, E.: An apparent discrepancy between chain length and electrophoretic mobility of restriction fragments: A case of human mitochondrial DNA. Human Genet. **75**: 73–74, 1987.

HORI, H. and SATOW, Y.: IRIS: Integrated RNA information for systematics. In "*Prospectives in Nucleic Acid and Protein Sequencing Data; Collection, Evaluation, Distribution*" (R. R. Colwell, ed.), Oxford Univ. Press, in press.

HORI, H. and OSAWA, S.: Origin and evolution of organisms as deduced from 5S ribosomal RNA sequences. Mol. Biol. Evol. **4**: 445–472, 1987.

HORI, H., MUTO, A., OSAWA, S., TAKAI, M. and KAWAMATSU, M.: Molecular evolution

of Turbellaria as deduced from 5S ribosomal RNA sequences. Prog. Zool. **36**: in press.

HORIKAWA, K., OTAFUJI, T., NAKAGAWA, R., SERA, N., KURODA, Y., OTSUKA, K. and TOKIWA, H.: Mutagenicity of dinitrofluoranthenes (DNFs) and their carcinogenicity in F344 rats. Mutation Res., **182**: 360–361, 1987.

IMAMURA, T.: Hemoglobinopathies. (in Japanese) In "*Practice of Internal Medicine*, 5th ed." (M. Abe *et al.*, eds), 557–569, Igaku-Shoin, Tokyo, 1987.

IMAMURA, T.: Thalassemia genes. (in Japanese) In "*Annual Review of Haematology* 1987" (F. Takaku *et al.*, eds), 48–57, Chugai-Igaku-Shoin, Tokyo, 1987.

IMAMURA, T.: Disorders of complement receptors in autoimmune diseases. (in Japanese) Immune Haematology **8**: 413–426, 1987.

IMAMURA, T.: Mendelism and clinical medicine. (in Japanese) Clinical Pediatrics **50**: 573–575, 1987.

ISHIHAMA, A., FUJITA, N. and NOMURA, T.: Promoter selectivity of *Escherichia coli* RNA polymerase. In "*RNA Polymerase and the Regulation of Transcription*" (W. S. Reznikoff, *et al.*, eds.), pp. 397–402, Elsevier Science Publ., New York, 1987.

ISHIHAMA, A. and NAGATA, K.: Viral RNA polymerases. In "*CRC Reviews in Biochemistry*", CRC Press, Florida, 1987.

ISHIHAMA, A., FUJITA, N. and GLASS, R. E.: Subunit assembly and metabolic stability of *E. coli* RNA polymerase. Proteins: Structure, Function and Genetics, **2**: 42–53, 1987.

ISHIHAMA, A., HONDA, A., NAGASAWA-FUJIMORI, H., GLASS, R. E., MAEKAWA, T. and IMAMOTO, F.: Multivalent regulation of the nusA operon of *Escherichia coli*. Mol. Gen. Genet. **206**: 185–191, 1987.

ISHIHAMA, A.: Transciption and RNA processing. (in Japanese) In "*Medical Molecular Biology*" (M. Muramatsu, ed.), 36–41, Nanko-do, Tokyo, 1987.

ISHIHAMA, A.: Mechanism of gene transcription. (in Japanese) In "*New Approaches in Life Sciences*" (The Org. Committee of Symp. on Univ. and Sci., ed.), 235–255, Shuppan-Kagaku, Tokyo, 1987.

ISHIKAWA, R., MORI, K., KINOSHITA, T. and MORISHIMA, H.: Expression pattern of isozyme genes in rice calli. (in Japanese) Memoirs of Fac. Agr., Hokkaido Univ. **15**: 371–379, 1987.

ITOH, N., YAMADA, H., KAZIRO, Y. and MIZUMOTO, K.: Messenger RNA guanylyltransferase from *Saccharomyces cerevisiae*: Large scale purification, subunit functions, and subcellular localization. J. Biol. Chem. **262**: 1989–1995, 1987.

JAIN, A. K. and TEZUKA, H.: Effect of age on micronucleus frequency induced by mitomycin C in ddY male mice. Curr. Sci. **56**: 894–895, 1987.

JAIN, A. K., TEZUKA, H., KADA, T. and TOMITA, I.: Evaluation of genotoxic effects of turmeric in mice. Curr. Sci. **56**: 1005–1006, 1987.

KADA, T. and SHIMOI, K.: Desmutagens and bioantimutagens. Their modes of action. BioEssays **7**: 113–116, 1987.

KIMURA, M., KALLIANPUR, G. and HIDA, T. (ed.): *Stochastic Methods in Biology* (Lecture Notes in Biomathematics 70). Springer-Verlag, Berlin, 1987.

KIMURA, M.: A stochastic model of compensatory neutral evolution. In "*Stochastic*

Methods in Biology" (M. Kimura, *et al.*, eds.), pp. 2–18, Springer-Verlag, Berlin, 1987.

KIMURA, M.: *Die Neutralitatstheorie der molekularen Evolution.* Verlag Paul Parey, Berlin und Hamburg, 1987.

KIMURA, M.: Molecular evolutionary clock and the neutral theory. J. Mol. Evol. **26**: 24–33, 1987.

KOJIMA, H., KONISHI, H. and KURODA, Y.: Combined effects of chemicals on mammalian cells in culture. Effects of order and intervals of administration of methyl methanesulfonate and ethyl methanesulfonate. Mutation Res., **182**: 364, 1987.

KOMIYAMA, K., ITO, K. and SHIMADA, Y.: Origin and development of the epicardium in the mouse embryos. Anatomy and Embryology **176**: 183–189, 1987.

KONDO, K., AOSHIMA, Y., HAGIWARA, T., UEDA, H. and MIZUNO, S.: Tissue-specific and periodic changes in the nuclease sensitivity of the fibroin gene chromatin in the silkworm *Bombyx mori*. J. Biol. Chem. **262**: 5271–5279, 1987.

KURODA, Y.: Antimutagenic mechanism of vitamin C and its derivatives in mammalian cells in culture. Mutation Res. **182**: 365, 1987.

LEE, W. H. and WATANABE, T. K.: Evolutionary genetics of the *Drosophila melanogaster* subgroup. I. Phylogenetic relationships based on matings, hybrids and proteins. Jpn. J. Genet. **62**: 225–239, 1987.

MATSUBAYASHI, T., WAKASUGI, T., SHINOZAKI, K., YAMAGUCHI-SHINOZAKI, K., ZAITA, N., HIDAKA, T., MENG, B. Y., OHTO, C., TANAKA, M., KATO, A., MARUYAMA, T. and SUGIURA, M.: Six chloroplast genes (*ndhA-F*) homologous to human mitochondrial genes encoding components of the respiratory chian NADH dehydrogenase are actively expressed: Determination of the splice sites in *ndhA* and *ndhB* pre-mRNAs. Mol. Gen. Genet. **210**: 385–393, 1987.

MATSUNAGA, E.: Hereditary diseases in Asia: Chairman's introduction. In "*Human Genetics*" (F. Vogel and K. Sperling, eds.), p. 539, Springer-Verlag, Berlin, 1987.

MATSUNAGA, E.: Incidence of genetic disease in Japan. In "*Human Genetics*" (F. Vogel and K. Sperling, eds.), pp. 540–546, Springer-Verlag, Berlin, 1987.

MATSUNAGA, E.: Possible effect of biotechnology development upon human gene pool. (in Japanese) In "*Biotechnology & Medical Care*" (F. Takaku, ed.), 57–71, Tokyo Univ. Press, 1987.

MATSUNAGA, E.: Statistics in human genetics, with special reference to the study of retinoblastoma. (in Japanese) In "*Statistics in Science*" (H. Akaike, ed.), 181–216, Kodansha, Tokyo, 1987.

MATSUNAGA, E.: Genetics and epidemiology of retinoblastoma. (in Japanese) Gan no Rinsho **33**(5): 507–513, 1987.

MINODA, K., HORAI, S., MATSUNAGA, E. and MOTEGI, F.: Esterase D and chromosome 13 in patients with retinoblastoma. (in Japanese) Rinsho Ganka **41**: 943–947, 1987.

MIYASHITA, N., MIGITA, S. and MORIWAKI, K.: Effects of H-2 complex and non-H-2 background on urethane-induced chromosomal aberrations in mice. Mutation Res. **176**: 59–67, 1987.

MIYASHITA, N. and MORIWAKI, K.: H-2 controlled genetic susceptibility to pulmonary adenomas induced by urethane and 4-nitroquinoline 1-oxide in A/Wy congenic strains.

Jpn. J. Cancer Res. (Gann) **78**: 494–498, 1987.

MIZUMOTO, K. and KAZIRO, Y.: Messenger RNA capping enzyme from eukaryotic cells. Prog. Nucl. Acid. Res. Mol. Biol. **34**, 1987. (in press)

MORISHIMA, H. and GADRINAB, L. U.: Are the Asian common wild-rices differentiated into the Indica and Japonica types? In "*Crop Exploration and Utilization of Genetic Resources*" (S. C. Hsieh, ed.), 11–20, Taiwan Prov. Taichung Dist. Agric. Imp. Stat., 1987.

MORIWAKI, K.: Genetic significance of laboratory mice in biomedical research. In "*Animal Models: Assessing the Scope of Their Use in Biomedical Research*" (J. Kawamata & E. C. Melby, eds.), pp. 53–72, Alan R. Liss, New York, 1987.

MORIYA, S., OGASAWARA, N., FUKUOKA, T. and YOSHIKAWA, H.: DnaA-boxes in the *oriC* region of the *Bacillus subtilis* chromosome are involved in regulation of chromosomal replication. Genetic and Biotechnology of Bacilli. (in press).

MORIYA, S., OGASAWARA, N., FUKUOKA, T. and YOSHIKAWA, H.: Regulation of initiation of the chromosomal replication by DnaA-boxes in the origin region of the *Bacillus subtilis* chromosome. EMBO J. (in press)

NAKASHIMA, H.: Genetic counselings. (in Japanese) JAMA **6**: 120–122, 1987.

NAWA, S., SANO, Y., YAMADA, M. and FUJII, T.: Cloning of the plasmids in cytoplasmic male sterile rice and changes of organization of mitochondrial and nuclear DNA in cytoplasmic reversion. Jpn. J. Genet. **62**: 301–314, 1987.

NITO, S., OKANIWA, A. and MORIWAKI, K.: Effects of H-2 histocompatibility gene complex on urethan and ethylnitrosourea (ENU)-induced malformations in mice. Cong. Anom. **27**: 61–68, 1987.

NOMURA, A., NEGISHI, K., HAYATSU, H. and KURODA, Y.: Mutagenicity of N^4-aminocytidine and its derivatives in Chinese hamster lung cells. Incorporation of N^4-aminocytidine into cellular DNA. Mutation Res. **177**: 283–287, 1987.

NOMURA, T,, FUJITA, N. and ISHIHAMA, A.: Expression of *leuX* gene in *Escherichia coli*. Regulation at transcription and tRNA processing steps. J. Mol. Biol. **197**: 659–670, 1987.

OHTA, T.: A model of evolution for accumulating genetic information. J. Theor. Biol. **124**: 199–211, 1987.

OHTA, T.: Simulating evolution by gene duplication. Genetics **115**: 207–213, 1987.

OHTA, T.: Very slightly deleterious mutations and the molecular clock. J. Mol. Evol. **26**: 1–6, 1987.

OHTA, T., WATANABE, M., SHIRASU, Y. and INOUE, T.: Post-replication repair and recombination in *uvrA umuC* strains of *Escherichia coli* are enhanced by vanillin, an antimutagenic compound. Mutation Res. (in press).

OHTSUBO, M., KAI, R., FURUNO, N., SEKIGUCHI, T., SEKIGUCHI, M., HAYASHIDA, H., KUMA, K., MIYATA, T., FUKUSHIGE, S., MUROTSU, T., MATSUBARA, K. and NISHIMOTO, T.: Isolation and characterization of the active cDNA of the human cell cycle gene (RCC1) involved in the regulation of onset of chromosome condensation. Genes and Development **1**: 585–593, 1987.

PARK, Y. H., HORI, H., SUZUKI, K. I., OSAWA, S. and KOMAGATA, K.: Nucleotide sequence of 5S ribosomal RNA from *Rhodococcus erythropolis*. Nucleic Acids Res.,

15, 365, 1987.

PARK, Y. H., HORI, H., SUZUKI, K. I., OSAWA, S. and KOMAGATA, K.: Phylogenetic analysis of the coryneform bacteria by 5S rRNA sequences. J. Bacteriol. **169**, 1801–1806, 1987.

SAKOYAMA, Y., HONG, K., BYUN, S. M., HISAJIMA, H., UEDA, S., YAOITA, T., HAYASHIDA, H., MIYATA, T. and HONJO, T.: Nucleotide sequences of immunoglobulin epsilon genes of chimpanzee and orangutan: DNA molecular clock and hominoid evolution. Proc. Natl. Acad. Sci. USA **84**: 1080–1084, 1987.

SANO, Y.: Gene regulation at the *waxy* locus in rice. Gamma-Field Symp. **24**: 63–79, 1987.

SATO, Y. I.: Character association patterns among Japonica rice varieties of Asian origin. In "*Crop Exploration and Utilization of Genetic Resources*" (S. C. Hsieh, ed.), 21–29, Taiwan Prov. Taichung Dist. Agric. Imp. Stat., 1987.

SATO, Y. I. and MORISHIMA, H.: Studies on the distribution of complementary genes causing F_1 weakness in common rice and its wild relatives. 2. Euphytica **36**: 425–531, 1987.

SERIZAWA, H. and FUKUDA, R.: Structure of the gene for the stringent starvation protein of *Escherichia coli*. Nucleic Acids Res. **15**: 1153–1163, 1987.

SHIMADA, Y., KOMIYAMA, M., SHIOZAKI, M., ISOBE, Y. and MASUKO, S.: Myogenesis *in vitro* as seen with the scanning electron microscope. Scanning Microscopy **1**: 1377–1386, 1987.

SHIROISHI, T., SAGAI, T. and MORIWAKI, K.: Sexual preference of meiotic recombination within the H-2 complex. Immunogenetics **25**: 258–262, 1987.

SHIROISHI, T., SAGAI, T., NATSUUME-SAKAI, S. and MORIWAKI, K.: Lethal deletion of the complement component C4 and steroid 21-hydroxylase genes in the mouse H-2 class III region, caused by meiotic recombination. Proc. Natl. Acad. Sci. USA **84**: 2819–2823, 1987.

SUH, D.-H. and MUKAI, T.: Mosaic lethal mutations as one of the syndrome of hybrid dysgenesis due to the P element in *Drosophila melanogaster*. Jpn. J. Genet. **62**: 51–58, 1987.

SUH, D.-H. and MUKAI, T.: Genetic variability due to mutation-selection balance in a northern population of *Drosophila melanogaster* in Japan. Jpn. J. Genet. **62**: 95–107, 1987.

SUZUKI, H., MORIWAKI, K. and NEVO, E.: Ribosomal DNA (rDNA) spacer polymorphism in mole rats. Mol. Biol. Evol. **4**: 602–610, 1987.

SUZUKI, H., KATO, J., SAKAGAMI, Y., MORI, M., SUZUKI, A. and HIROTA, Y.: Conversion of the α component of penicillin-binding protein 1b to the β component in *Escherichia coli*. J. Bacteriol. **169**: 891–893, 1987.

TACHI-SHINKAWA, K., KURODA, Y., MORIMOTO, K. and KOIZUMI, A.: Enhancing effects of cytosine arabinoside on ethyl methanesulfonate-induced 6-thioguanine resistance mutations in Chinese hamster V79 cells. Mutation Res. **191**: 37–40, 1987.

TAKAHASHI, T., MATSUDAIRA, Y., OBATA, Y. and MORIWAKI, K.: An autoreactive H-2-specific monoclonal antibody with allospecificity. Immunogenetics **26**: 105–106, 1987.

TAKAHATA, N.: On the overdispersed molecular clock. Genetics **116**: 169–179, 1987.

TAKANO, T., KUSAKABE, S. and MUKAI, T.: The genetic structure of natural populations of *Drosophila melanogaster*. XX. Comparison of genotype-environment interaction in viability between a northern and a southern population. Genetics **117**: 245–254, 1987.

TAKEUCHI, K., NAGATA, K. and ISHIHAMA, A.: *In vitro* synthesis of influenza viral RNA: Characterization of an isolated nuclear system that supports transcription of influenza viral RNA. J. Biochem. **101**: 837–845, 1987.

TAKEUCHI, M., HARA, M., INOUE, T. and KADA, T.: Adsorption of mutagens by refined corn bran. Mutation Res. (in press)

TOMITA, T., TANAKA, T., KOGA, A., NAKAMA, T., KONDO, K., YAKENO, Y. and MUKAI, T.: Strain difference in homozygous load due to induced mildly deleterious mutations in *Drosophila melanogaster*. Proceedings of the Japan Academy. **63**: 151–154, 1987.

WATANABE, T., MIYASHITA, N., MORIWAKI, K. and HILGERS, J.: Evolutionary relationships between laboratory mice and subspecies of *Mus musculus* based on the genetic study of pancreatic proteinase loci, *Prt*-1, *Prt*-2, *Prt*-3, and *Prt*-6. Biochem. Genet. **25**: 239–251, 1987

WU, C., WILSON, S., WALKER, B., DAWID, I., PAISLEY, T., ZIMARINO, V. and UEDA, H.: Purification and properties of *Drosophila* heat shock activator protein. Science **238**: 1247–1253, 1987.

YAMAMOTO, T., GOJOBORI, T. and YOKOTA, T.: Evolutionary origin of pathogenic determinants in enterotoxigenic *Escherichia coli* and *Vibrio cholerase* 01. J. Bact. **169**: 1352–1357, 1987.

YOKOYAMA, S. and GOJOBORI, T.: Phylogeny and molecular evolution of the human AIDS viruses LAV, HTLV-III, and ARV. J. Mol. Evol. **24**: 330–336, 1987.

YOKOYAMA, S., MORIYAMA, E. N. and GOJOBORI, T.: Molecular phylogeny of the human immunodeficiency and related retroviruses. Proc. Jap. Acad. **63(B)**: 147–150, 1987.

YONEKAWA, H., MORIWAKI, K., GOTOH, O., MIYASHITA, N., MATSUSHIMA, Y., SHI, L., CHO, W. S., ZHEN, X.-L. and TAGASHIRA, Y.: Hybrid origin of Japanese mice "Mus musculus molossiums"; Evidence from restriction analysis of mitochondrial DNA. Mol. Biol. Evol. (in press)

YONEKAWA, H. and FISCHER LINDAHL, K.: Searching for the molecular basis of a mitochondrial transplantation antigen. Genetics **116**: s41, 1987.

YONEKAWA, H. and FISCHER LINDAHL, K.: Molecular evolution of mtDNA among closely related Mus species. Abstracts of papers presented at the 1987 meeting on molecular biology of mitochondria and chloroplasts. p. 205. Cold Spring Harbor Laboratory, 1987.

ABSTRACTS OF DIARY FOR 1987

Biological Symposium

257th	meeting	Dec. 12 1986	Is the classic case of natural selection a classic case of another kind? (D. Lambert)
258th	—	Dec. 19	Adaptive evolution at the molecular level (M. Nei)
259th	—	Mar. 18 1987	Reduction of acrosomal protenase level in mouse spermatozoa caused by partial deletion of Y-chromosome (J. Styrna)
260th	—	Apr. 9	MHC polymorphism (J. Klein)
261st	—	May 25	Molecular biology of T cell lymphokines: A model system for proliferation and differentiation of mammalian cells (K. Arai)
262nd	—	May 25	A novel method for transfection of DNA-protein complex to eukaryotic cells (U. Wienhues)
263rd	—	May 29	Consequences on brain function of in-utero exposure to atomic bombing in Hiroshima and Nagasaki (W. J. Schull)
264th	—	Jun. 20	Genetics and development of mice (Hee-Sup Shin)
265th	—	Oct. 27	Phylogenetic origin of t-haplotypes specific markers (G. Gachelin)
266th	—	Nov. 17	High natural radiation area in Brazil (A. Freire-Maia)
267th	—	Nov. 30	Protein processing in picona virus-infected cells (B. D. Korant)
268th	—	Dec. 4	Identifying domains in protein sequences (W. C. Barker)

Mishima Geneticists' Club

319th	meeting	Feb. 5	Modulation of the MHC class I gene expression and tumorgenesis (K. Tachida)
320th	—	Apr. 17	Mammalian DNA replication (F. Hanaoka)
321st	—	May 20	Regulation of the insect homeotic genes (H.

			Ueda)
322nd	—	Jun. 9	Relational gene library of E. coli genome (Y. Ohara)
323rd	—	Jun. 25	Chromosome translocations in human cancer cells (Y. Tsujimoto)
324th	—	Jul. 3	Theoretical study of protein conformational changes and dynamics by computer simulation (N. Go)
325th	—	Jul. 10	Mechanisms of DNA replication in the yeast Saccharomyces cerevisiae (A. Sugino)
326th	—	Jul. 24	Organization and expression of Marchantia polymorpha chloroplast genome (K. Umezono)
327th	—	Aug. 26	Induction of targeted point mutation by a site-specific acetylaminofluorene adduct to DNA in bacteria and mammalian cells (M. Moriya)
328th	—	Oct. 2	Replication initiation of plasmid pSC101 (K. Yamaguchi)
329th	—	Oct. 2	Roles of transcription in initiation of DNA replication: a case of plasmid ColEI (H. Masukata)
330th	—	Nov. 27	Probable heterosis and functional differences of isozymes in aquatic animals (K. Fujino)
331st	—	Dec. 10	A cell surface marker protein of murine embryogenesis, fotomodulin, and its application to molecular embryogenetic study (M. Imada)
332nd	—	Dec. 18	Permanency of response to selection in finite populations and effect of identity disequilibrium on it (H. Tateda)

FOREIGN VISITORS IN 1987

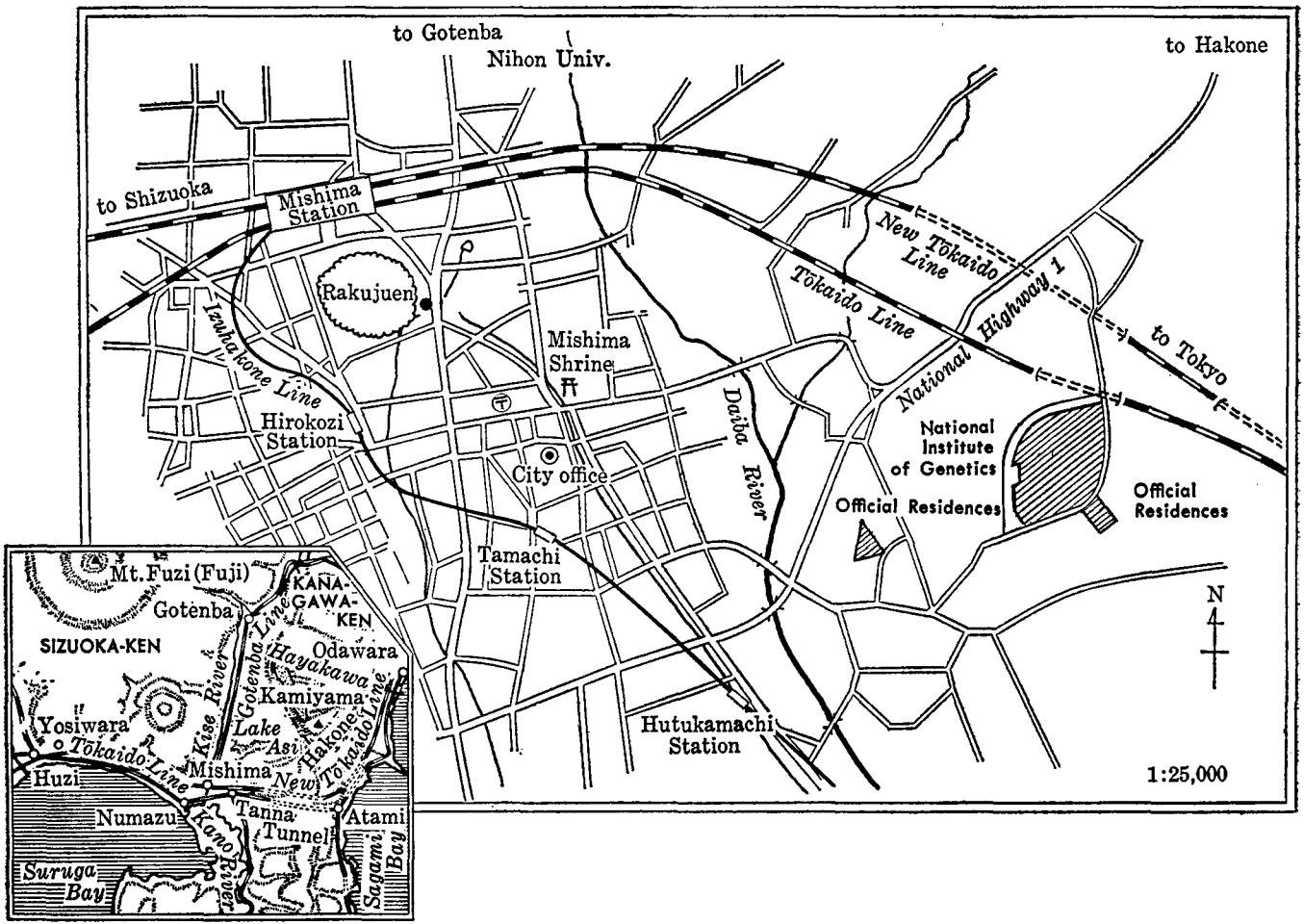
April 9, 1984–	Barbier, Pascale, Université des Sciences et Techniques du Languedoc Montpéllier, France
January 30, 1986–January 29, 1987	Styrna, Jozefa, Jagiellonian University, Poland
April 22, 1986–April 19, 1987	Hong, Nguen Xuan, Hanoi Universtiy, Vietnam
January 16	Chen, Zheng Hua, Genetics Institute, Chinese Academy of Sciences, China
January 27	Wu, Xiang Lin, Lanzhou Institute of Biological Products, Ministry of Public Health, China
February 7–8	Bodmer, Walter F., Imperial Cancer Research Fund Laboratories, U.K.
February 7–8	Yoo, Ick-dong, Genetic Engineering Center, Korea Advanced Institute of Technology, Korea
March 14 March 17–19	Brenton, Nick, BBC Open University Production Centre, U.K.
March 18	Styrna, Jozefa, Jagielonian University, Poland
April 1–	Suh, Dong Sang, Genetic Engineering Center, Korea Advanced Institute of Technology, Korea
April 9	Klein, Jan, Max-Planck-Institut für Biologie, West Germany
May 6	Sun, Chong Rong, Fudan University, China
May 6	Lu, Qun, Institute of Genetics, Fudan University, China
May 25	Arai, Ken-ichi, DNAX Research Institute of Molecular and Cellular Biology, U.S.A.
May 25	Wienhues, Ursula, University of Cologne, West Germany
May 27	Pardo, Daniel, French Embassy, Tokyo
May 29	Schull, William Jack, Radiation Effects Research Institute, Hiroshima (University of Texas, U.S.A.)
June 20	Shin, Hee-Sup, Whitehead Institute, MIT, U.S.A.
June 25	Tsujimoto, Yoshihide, The Wistar Institute, U.S.A.

July 10	Sugino, Akio, Laboratory of Genetics, NIEHS/NIH, U.S.A.
July 22	Grote, John, The British Council, Tokyo
August 4–	Tang, Ling Hua, Institute of Food Crops, Jiangsu Academy of Agricultural Sciences, China
September 1–	Kim, Sam-Eun, Sericultural Experiment Station, Rural Development Administration, Korea
October 9	Fouden, Leslie, Rothampsted Experimental Station, Agricultural and Food Research Council, U.K.
October 27	Gachelin, Gabriel, Institut Pasteur, France
November 10	Pan, I-Hung, National Taiwan University, Taiwan
November 14–22	Yi, Hao Xiong, Jiang Xi University, China
November 16	Shao, Qi Quan, Institute of Genetics, Academia Sinica, China
November 16–17	Freire-Maia, Ademar & Dertia V., Sao Paulo State University, Brazil
November 17–22	Nasution, Rusdy E., Bogor Botanical Garden, National Institute of Biology, Indonesia
November 30	Korant, Bruce D., E. I. du Pont de Nemours & Co., U.S.A.
December 4	Barker, W. C., Georgetown University Medical Center, U.S.A.
December 22–	Wu, Xiao Mei & Zhao, He, Lanzhou Institute of Biological Products, Ministry of Public Health, China

AUTHOR INDEX

国立遺伝学研究所年報　第38号
昭和63年8月10日　印刷
昭和63年9月20日　発行

発行者　松　永　　英
国立遺伝学研究所内
編集者　名和三郎・藤沢敏孝
国立遺伝学研究所内
印刷者　笠　井　康　弘
東京都新宿区高田馬場 3-8-8
印刷所　株式会社 国際文献印刷社
東京都新宿区高田馬場 3-8-8
発行所　国立遺伝学研究所
〒411　静岡県三島市谷田 1111
電　話　代表 (0559) (75) 0771


to Gotenba
Nihon Univ.
to Hakone
to Shizuoka
Mishima Station
Rakujuen
Izuhakone Line
Mishima Shrine
New Tōkaido Line
Tōkaido Line
Highway 1
to Tokyo
National
Hirokozi Station
City office
Daiba River
National Institute of Genetics
Official Residences
Official Residences
Tamachi Station
Hutukamachi Station
N
1:25,000
Mt. Fuzi (Fuji)
KANA-GAWA-KEN
Gotenba
Gotenba Line
SIZUOKA-KEN
Kise River
Hayakawa
Odawara
Kamiyama
Yosiwara
Lake Asi
Hakone Tōkaidō Line
Tōkaido Line
Mishima
New
Huzi
Numazu
Kano River
Tanna Tunnel
Atami
Suruga Bay
Sagami Bay